SOME FACTORS IN THE LOCALISATION OF DISEASE IN THE BODY

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To
G. M. B.

PREFACE

THE production of a book which deals with some of the less widely known, though fundamental, phenomena of life, requires no apology; though the incompleteness of the present volume demands some explanation. At a very early stage of the work the author realised that he was in no way qualified to deal single-handed with a subject so complex as the present one, involving, as it does, the application of electricity and other branches of physical science to the interpretation of biological events. Consequently he has had to restrict himself mainly to the collection of empirical observations in this field. Much biological and experimental work which is within the writer's capacity and which, in his opinion, ought to be done, is still waiting to be carried out; and the relevant literature has as yet been inadequately studied. For these several deficiencies there is one excuse, namely, the breadth of the subject. The processes involved, which the author has attempted to integrate into a system, extend through the entire domain of pathology, encroach upon the province of physiology, and call for consideration in the diagnosis and treatment of disease. The author felt that to set out the observations which have already been made, and to found upon them some suggestions which may prove useful to more specialised minds, would be better than to attempt vainly to acquire a personal mastery of the whole complicated problem.

Accepting the principles submitted in the following pages, it would be possible to advance fresh hypotheses as to the causation, investigation, and treatment of many illnesses—for example, epilepsy, bronchitis, puerperal sepsis, chronic arthritis, vaccinial encephalitis—while additional light might be thrown upon certain physiological events, among which are the fertilisation of the ovum and the adaptation of structures in response to stress. As a rule, the writer has refrained from introducing such speculative matter, because the bearings of the general theme are to a large degree self-evident. When a speculation has been ventured upon in the text, the intention has been rather to give life to the dry bones of fact than to anticipate what may in the future, but cannot at the present time, be regarded as established truth.

The notes and observations upon which this book is based are largely the outcome of an inquiry into the localisation of cancer carried out at the Research Institute of the Cancer Hospital (Free), to the authorities of which the writer takes this opportunity of tendering his thanks for the facilities provided. To Professor Kennaway, Director of the Research Institute, he is very deeply indebted for technical information and never-failing encouragement. To Mr. Mayneord and his other colleagues, who have always been ready to supply advice and information, and to the laboratory attendants whose loyal co-operation has been of great assistance, the author wishes to express his sense of obligation and gratitude.

H. B.

LONDON,
April, 1932.

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PART I

LOCALISATION FROM THE BLOODSTREAM OF COLLOIDAL AND OTHER MATTERS, INCLUDING BACTERIA AND CELLS

CHAPTER I

INTRODUCTION

The localisation of disease is often the first step toward recovery. When aggressive agents have entered the body the immediate safety of the individual usually is ensured if the aggressors can be retained or collected into a circumscribed region where the integrity of the tissues is not a vital necessity, and where the powers of resistance may be exercised in concentrated force. In this essay an attempt has been made to elucidate the circumstances in which such segregation and retention come about, and to determine by what artificial means these defensive processes may be assisted.

In order to obtain firm ground for subsequent discussion, the writer has collected examples of the localisation of various substances including colloids, larger inorganic particles, viruses, and micro-organisms from the bloodstream; and these examples, derived both from clinical and experimental work, have been assumed to be a strong enough foundation for the general principles embodied in subsequent pages.

The process of localising colloids and coarsely particulate matter from the blood is composed of three phenomena: (1) an increase of the permeability of the capillary endothelium, (2) the escape of substances from the bloodstream by traversing this altered endothelium, and (3) the arrest and temporary confinement of these substances within the extravascular tissues where they

have arrived. Together these three components, so far as they are understood, give a scientific meaning to the phrase "locus minoris resistentiæ"—a phrase which, perhaps, may be regarded as not too fortunate, since it applies to the scenes of conflict where most of our successes against diseases have been won; for the same influences which lead to the segregation of a pathogenic agent into a definite field lead also to the accumulation there of the defensive forces of the host.

There is another aspect of the matter, inasmuch as we have to consider those cases of localisation which are not favourable to the health of the individual. For example, it can hardly be regarded as satisfactory from the hygienic point of view when toxins have free access to the central nervous system or pathogenic bacteria form colonies in the valves of the heart, where irreparable damage may be done even though the causative agent be ultimately destroyed; nor is it helpful to the newly delivered woman if chance bacteria travelling in her bloodstream become localised in the placental site, where there is a favourable nidus for their multiplication.

At first sight the passage of colloidal and other particles through the vascular endothelium might be regarded merely as an attribute of inflammation. When the subject is examined with more exactitude it becomes manifest that such permeation may occur in conditions other than inflammatory. Thus it is a pronounced and widely spread phenomenon of general shock and of the antibody-antigen reaction; it is a normal occurrence in the healthy placenta and the liver, in clean granulating wounds, in the brain following concussion, and in certain special forms of poisoning. The endothelium of the capillaries in and about

a scar may remain more permeable than normal for long periods of time in the absence of any ordinary evidence of inflammation, and a similar statement is applicable to some nævi and to the dusky areas of a mottled skin.

In this connection we must consider the case of endothelial cells which have been inflamed but have failed to make a complete recovery, remaining unduly permeable, perhaps for long periods of time, though quite removed from the agent which injured them. The possibility of such a condition of imperfect recovery has been brought to light by Osterhout's work on the electrical conductivity of cells, and is, the author believes, exemplified by the condition described by Lewis[2] as irresponsiveness, in which the small vessels of the affected tissue fail to react in the ordinary way to applications of histamine or adrenalin. Such an imperfect recovery from inflammation, accompanied by a continuing permeability of capillary endothelium, appears to play an important part in the localisation of noxious agents from the blood, and yet to describe it as inflammation would be inaccurate. The excessive permeability, also, of newly formed vessels may perhaps be attributed to their immaturity, but can hardly be ascribed to inflammation.

Again, the walls of capillary vessels, when submitted to ischæmia for a sufficient period, will become incontinent, and so, too, will those which are subjected to the stagnant blood of venous obstruction. Nor are metabolites the only poisons which, acting from within the vessels, may render their walls more permeable than normal; and although the direct response of endothelium to a circulating poison may perhaps be de-

scribed as inflammation, the response is simpler than the complicated reaction of the tissues in bulk to which the term "inflammation" is commonly applied.

An increased concentration of hydrogen ions in the circulating blood or in the extravascular tissues appears to be a potent and, it may be, a physiological regulator of the permeability of capillary endothelium. Apparently a hydræmia, such as may accompany some forms of nephritis or any other state in which a reduction of the circulating proteins occurs, may of itself be sufficient to bring about an abnormal permeability of the capillary walls, and a similar cellular defect may accompany amyloid and perhaps other forms of cellular degeneration.

Even when an increase of permeability is due to frank inflammation the amount of permeation is not in proportion to the degree of the inflammatory process, being often most pronounced—over an extended period of time—with the lesser grades.

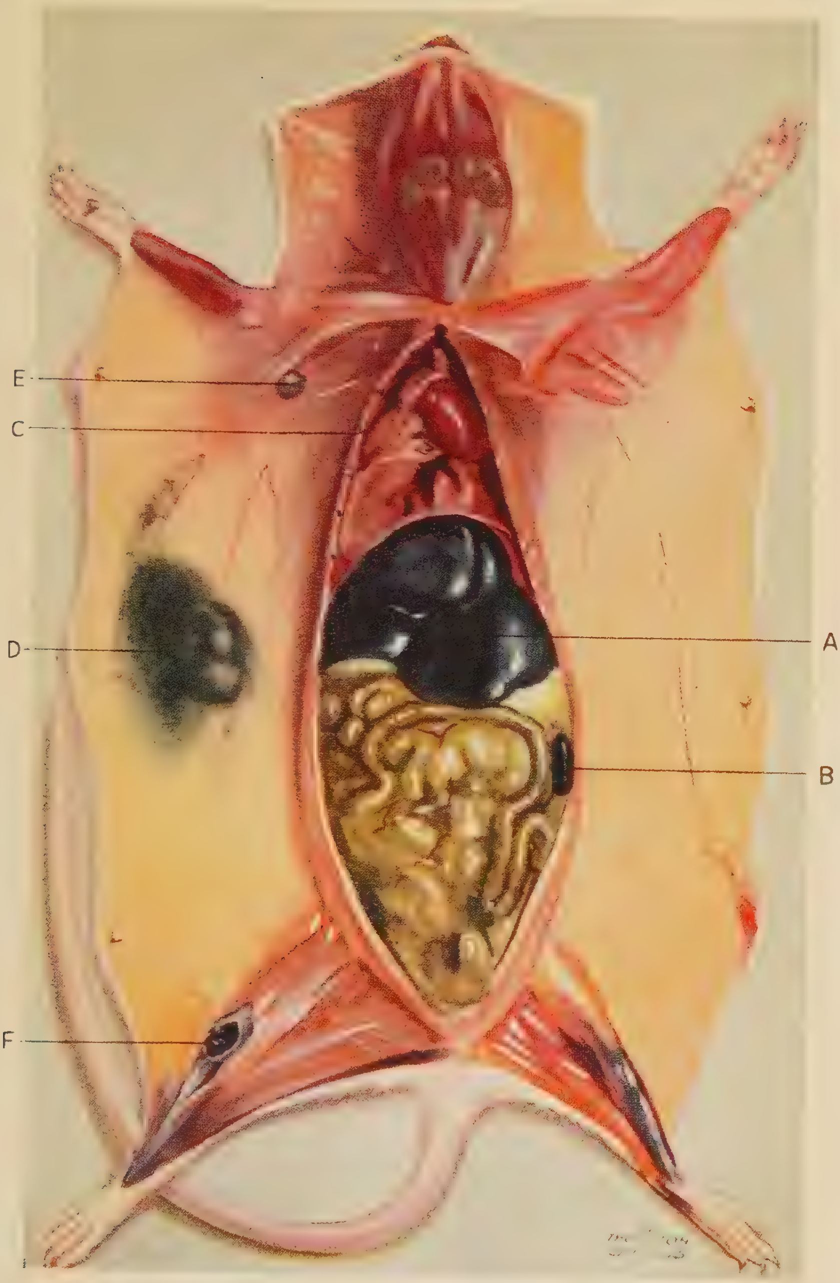
The first requirement, therefore, is to provide a special name for this physiological entity in order to facilitate its discussion. "Increased permeability of the bloodvessels" is a term in common use; against it is the fact that there are regions of the body where the walls of the bloodvessels are constantly permeable to colloids and their transit is in these regions a normal function of everyday life, and to apply the term "increased permeability" in these cases would be incorrect and confusing. Again, the word "permeation" is already in established use to describe the spread of malignant growths along the lymphatic channels, and this alone would almost prohibit the use of the word to describe the escape of colloids and particles from the blood, unless we were to use the phrase "increased

permeability of the walls of the bloodvessels," which is clumsy and still not free from objection. For such reasons I appealed to Sir D'Arcy Power for a name, and he has kindly suggested "Diapiresis," together with some alternatives. The word is derived from the Greek διαπείρειν (to drive through), and seems to be admirable, being free from unnecessary or undesirable implications. By diapiresis is meant the passage of colloidal or other particles of suspended matter, including bacteria and blood cells, through the unbroken walls of the bloodvessels.

Before considering the phenomenon in detail, it is necessary to know the normal fate of foreign colloids and suspended particles which have been introduced into the circulating blood. The disposal of these will be dependent primarily upon the electrical charge which they carry; and the following remarks are applicable only to those matters which, when present in the blood, are electronegative. As most of the substances to be considered—blood cells, bacteria, proteins, and other colloids—are negatively charged when carried in the blood, this method of dealing with the subject maintains a proper proportion. The fate of electropositive substances when introduced by artificial means will be mentioned briefly upon another page.

In the absence of any local inflammatory or other condition entailing diapiresis, electronegative foreign substances—whether in colloidal form or not, and whether organised or unorganised—will be removed speedily from the bloodstream by the phagocytic cells which line the sinusoidal blood spaces of various internal organs, and particularly those of the liver, the bonemarrow, and the spleen (Plate I.).

In the presence of inflammation and certain other local conditions an important modification of the normal process of disposal comes about. The endothelial cells of the capillary bloodvessels of the affected tissue, which in normal circumstances neither ingest fine particles nor allow them to escape from the circulation, except perhaps in minute quantity, now appear to become phagocytic and certainly become traversed by foreign colloids and other particles which, so long as the special conditions exist, pass with relative freedom out from the bloodstream and enter the surrounding extravascular tissues. It is credible that some true phagocytosis by the endothelial cells accompanies this diapiresis. This was the explanation adopted at the time by those who were the first to observe the presence in endothelial cells of Indian ink and other foreign substances which had been introduced into the bloodstream. On the other hand, it may be that the presence of granules of Indian ink or other detectable particles or colloids in the capillary endothelium of inflamed vessels after intravascular injection, as demonstrated by Mallory,[1] Foot, McJunkin,[1,2] and Hertzog, is due merely to the transit of these granules through the endothelial cells, and not to active phagocytosis as ordinarily understood. Indeed, it seems now to have been satisfactorily established by the work of Florey,[1] Lang, and Stilwell that phagocytosis is not the correct explanation in most instances. Florey has shown that, under the stress of inflammation, such a transit through the endothelial cytoplasm—though not through the nuclei—does in fact take place. By perfusing the intestinal bloodvessels of rabbits first with starch and afterwards with formalin and iodine



Localisation of Indian ink in reticulo-endothelial organs and inflamed tissue.

Inflammation was caused in a grafted sarcoma in a rat by an injection of agar. Four hours later Indian ink was given intravenously. The carbon particles have become localised in the liver (A), spleen (B) and bone-marrow (C) and (F), and also in the inflamed tissue (D) and in a lymphatic node (E). The lower end of the right femur (F) has been opened.

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he demonstrated the transit of starch through the endothelial cells. Similarly he perfused a dog's mesenteric vessels with potassium ferrocyanide and iron ammonium citrate for four minutes, and then with a 10 per cent. solution of formalin acidified with hydrochloric acid, and in sections made subsequently, he was able to demonstrate the presence of Prussian blue precipitated within the cytoplasm of the endothelial cells, the nuclei being free from any of this deposit.

Lang inserted small, sterile pieces of sponge soaked in lecithin into the subcutaneous tissue of rabbits, or, as an alternative, injected some yolk of egg into the same situation. Having initiated an aseptic inflammation in these ways, he gave intravenous injections of 5 c.c. of Indian ink with 5 c.c. of distilled water and killed the animals at varying intervals afterwards. Thus he was able to observe in the earliest stage carbon particles adhering to the inner surface of the capillary endothelium of the inflamed tissue. Later, carbon particles could be seen within the endothelial cytoplasm, and at the end of ten hours some of the particles had already completely traversed the capillary wall and become ingested by the perivascular macrophages. Stilwell made direct observations on the living capillaries of a frog's tongue which had been irritated by an injection of egg yolk. The frog was anæsthetised with urethane and Indian ink was injected into the femoral vein. It was possible in this way to watch the whole process of diapiresis—the arrest of the particles of Indian ink on the lining of the capillary, their slow passage through the endothelial cytoplasm, their escape into the perivascular spaces, and their phagocytosis by the tissue macrophages: these extravascular cells being

seen well charged with carbon at the end of five or six days after the injection.

A chapter will be devoted to the mechanisms of vascular permeation toward the end of the book, but it was thought that a preliminary explanation here might make the writer's purpose more clear to any reader who happened to have the patience to read the ensuing pages composed of examples collected from medical and physiological literature, and checked to some extent by clinical and laboratory experience.

CHAPTER II

INFLAMMATION

SEEING that inflammation is a potent and common cause of increased permeation of the capillary walls, it will be of advantage to discuss some of its characteristics now, so as to clarify subsequent statements.

Since Waller in 1846, completing the pioneer work of Addison of Malvern, and of Wharton Jones, established the fact that transudation and diapedesis occurred in response to irritation, many new methods have been applied to the study of inflammation, and numerous additional data have been obtained. Out of this mass of observation two ideas of especial import have arisen: one (*a*) is the concept of inflammation as a fundamental and elementary response of the living cell to injury, a response which in the higher multicellular organisms involves secondary features including those vascular phenomena which at one time were looked upon as the essential components of the inflammatory process; and the other (*b*) is an understanding that the secondary features of a response to irritation may vary in accordance with the particular character, intensity, and duration of the stimulus, the nature of the tissue undergoing stimulation, and the concomitant circumstances.

The only feature common to all cases is the individual cellular reaction against an adverse influence.

This essential factor of inflammation consists in the main of an increased permeability and electrical conductivity of the injured cell, whereby substances can

more freely enter the cell substance or escape therefrom. And it is necessary to bear in mind, whenever the complicated phenomena of inflammation, as it occurs in the higher organisms, are under consideration, that every cell coming into contact with an injurious agent will become more permeable, so that substances which in health are retained within the cell will exude into the surrounding medium, while other substances to which the cell is normally impermeable may now gain access to its interior. The capillary endothelium forms no exception to such a rule. Indeed, the secondary reactions of inflammation occurring in multicellular organisms must be regarded also as arising directly or indirectly from this fundamental and strictly local cellular response. It follows that when the complicated events of inflammation as seen in the vertebrata are under consideration the primitive cellular reactions which are always present must be regarded separately from the secondary and non-essential phenomena which may or may not be superimposed.

In an acute inflammation produced by a sufficiently strong stimulus the following stages may be seen: a brief pallor of the irritated tissue due to arteriolar constriction, soon followed by a dilatation of the arteries and veins leading to an increased velocity of circulation through the capillaries, which then gradually become dilated. Coincidently with the capillary dilatation the velocity of circulation diminishes, owing, as Lister expressed it, to stickiness affecting the vessel walls and the blood corpuscles. In the venules the zone of plasma which, so long as the circulation continues to be brisk, separates the axial stream of red cells from the vessel wall, gradually, as the bloodstream slows

down, comes to contain more and more white cells, which adhere to the endothelium. Accompanying this "pavementing" of the walls of the small vessels by leucocytes is an increase of permeability of the vascular endothelium, which now allows blood plasma to escape from the blood channels into the surrounding tissue spaces, while diapedesis of the white cells begins. If the inflammation is of sufficient severity the blood-stream continues to slow down until it is completely arrested, and the dilated capillaries become filled with red cells so closely packed together as to appear a homogeneous mass, every white cell having undergone diapedesis and all the plasma having exuded. After a while this condition of stasis may undergo resolution. The arrested corpuscles at the venous ends of the capillaries becoming detached are carried away in the bloodstream, and gradually this detachment proceeds along the capillary towards its arteriolar end until the blockage has been removed and a flow of blood is resumed.

Of the foregoing vascular changes the only essential ones are the stickiness of the capillary endothelial cells and the blood cells, and the increased permeability of the former. These are the invariable responses to irritation; in certain conditions any of the other features may be absent, with the exception, perhaps, of some degree of capillary dilatation. It seems clear that endothelium which is so much altered in its molecular constitution as to permit the passage of colloids and blood cells through its cytoplasm is unlikely to exercise by its tone any considerable control over the vascular calibre. In other words, it is hard to imagine that active constriction can occur in capillary vessels which

are under the influence of irritation. Nevertheless, if dilatation of the inflamed capillaries be regarded as being up to a point a passive condition, consequent upon arterial hyperæmia or other causes, then the absence of such dilatation is quite understandable even in the presence of inflammation. For it is possible to have inflammation without arterial hyperæmia. Lister himself noticed that applications of capsicum produced stasis in the capillaries of the web of a frog's foot without causing any capillary dilatation, and Kulisch observed a comparable phenomenon on applying cantharides to the skin of African hairless dogs. Hoff noticed that wheals could be raised in patients with factitious urticaria even when the skin had been rendered pale by a previous injection of adrenalin, and Tannenberg watched the occurrence of reversible stasis in the presence of arteriolar and capillary constriction produced by barium chloride. Hirschfelder found that although a preliminary vaso-constriction of the vessels of a rabbit's conjunctiva by means of cocaine checked to some extent the œdema which resulted from the application of mustard oil, it did not prevent the capillaries from becoming more permeable than normal, for trypan blue given intravenously still resulted in selective staining of the inflamed tissue.

To say that stasis, hyperæmia and so forth are not essential elements of inflammation, is not to affirm that they are without importance. These secondary responses may be the arbiters of death or survival in the conflict of an individual against bacterial infection. Nevertheless they are not the essential factors of inflammation, and confusion will be avoided by carrying this fact in the mind. The parts they

play will be considered in detail in a subsequent chapter.

That the degrees and results of inflammation vary greatly in accordance with the intensity and duration of the irritation is obvious. For example, the wheal that follows a stimulus of short duration applied to the skin is almost certainly unaccompanied by diapedesis, because the inflammatory response comes to an end before a sufficient interval of time has elapsed; for the escape of leucocytes is a slower process than the escape of plasma. Moreover, inflammations differ not only in duration and severity, but they differ also in kind; while one irritant will bring about a local concentration of polymorphonuclear leucocytes, another will result in an exudate consisting in the main of mononuclear lymphocytes and macrophages. Such differences may be of considerable therapeutic import (p. 235).

Yet another feature of inflammation has to be borne in mind, namely, that the injured cell, whether endothelial or otherwise, although surviving the injury, may not invariably make a perfect recovery, but may continue to be unduly permeable. Reference has been made already to this matter, but it appears to play such an important part in the localisation of disease that the repetition will perhaps be forgiven.

A few remarks may be made here concerning the escape of blood corpuscles and concrete particles of matter through the walls of the bloodvessels in inflammation. The very term "diapedesis" and the knowledge that leucocytes are endowed with motility have combined to bring about a misconception concerning the escape of these white corpuscles from the bloodvessels, which even today is not infrequently

attributed largely to their own powers of locomotion. Fifty years ago Cohnheim gave reasons for stating that diapedesis was a passive process, independent of any spontaneous activities of the leucocytes themselves. He found that if, while diapedesis were in progress, the arterial blood supply to the tissues under observation were arrested, all further emigration ceased instantaneously and completely. It is to be remembered too that leucocytes are not the only visible forms of matter that traverse the inflamed capillary walls, red cells, bacteria, and particles of inorganic matter being subject to the same transportation; and it is inherently probable that the forces which control the transport of other bodies will regulate largely if not entirely that of the leucocytes also.

That an advanced degree of endothelial permeability may be present without the accompaniment of diapiresis (as I have suggested the process should be called) is so obvious as hardly to need statement. For it does not follow that, whenever an increase of the permeability of the capillary walls is present, all the other conditions bringing about diapiresis will be present also.

The size of particles appears by direct observation to play a part in the speed with which they traverse the endothelial cytoplasm; for colloids pass through rapidly, while leucocytes require a much longer period. Clinical evidence, too, suggests that the readiness with which particles and organised bodies become localised in a tissue under the influence of irritation depends largely upon their size. Thus pigments and viruses are localised more certainly by inflammation, and by lesser degrees of it, than are required for the localisation of bacteria. There is reason for suspecting that the

smaller the virus the more readily will it become localised; thus the agent of foot-and-mouth disease, which is said to be the smallest known virus, will become segregated and therefore will cause lesions in tissues submitted to the mere physiological stresses accompanying mastication and the maintenance of a normal posture. Experimentally, also, it is found that the finer colloidal particles are more readily localised than the coarser suspensions of carbon or mercuric sulphide; and for the deposition of these substances in the extra-vascular tissues—which is what the term "localisation" includes—the substances must pass through the vascular endothelium. The effective dimensions of a body as they regulate its passage through an adaptable filter, as it were, apparently do not depend upon the size of the migrating mass as displayed to the eye; for by distortion some bodies may be enabled to pass through spaces which are minute in comparison with their undistorted outlines. This is the case with leucocytes and some other organised structures. The plausible suggestion has been made that the peculiar shape of the nucleus of the polymorphonuclear leucocyte is a special adaptation to facilitate the escape of the cell from the bloodvessels in inflammation.

The relatively large *Treponema pallidum* is said to traverse a porcelain filter whose pores are fine enough to retain most bacteria, and it seems possible that the great readiness with which the lesions of syphilis become localised in inflamed tissue may be explained on these lines, although reference must be made here to an alternative explanation to the effect that the treponema of syphilis has a life-cycle during part of which it exists in an ultramicroscopic form.

Summary.

(1) Inflammation is a common, though not the only, cause of increased endothelial permeability.

(2) The essential feature of inflammation is an increased permeability of the injured cell.

(3) The secondary manifestations of inflammation as seen in the higher animals vary widely in accordance with the duration, degree, and nature of the irritant.

(4) A cell, though surviving an injury, does not always recover completely; it may remain in a condition of abnormal permeability.

(5) Diapedesis is a passive process, being independent of activity on the part of the leucocytes.

(6) Not only blood cells, but inert particles also, are transported through the capillary endothelium under the influence of inflammation.

CHAPTER III

PERMEATION OF THE CAPILLARY ENDOTHELIUM BY NORMAL PROTEINS

SEEING that the localisation of proteins in the tissues from the bloodstream necessitates their passage through the walls of the bloodvessels, the conditions in which such transudation occurs will be the principal theme of this chapter; the arrest of these transuded proteins in the extravascular positions at which they have arrived will be considered separately in Chapter XIII.

The escape of plasma from the capillary bloodvessels as an accompaniment of inflammation was noticed by the earliest observers of diapedesis, who remarked upon the capacity of the exuded fluid to form clot. Since then much experimental inquiry has taken place concerning the formation of lymph, the occurrence of œdema, the transudation of plasma in shock, the whealing of skin in response to irritation, and other phenomena which involve the passage of normal proteins from the bloodstream into the tissues. A brief review of some of this work may be of interest. To preserve chronological order in such a review will not be easy unless the main features of lymph production as understood today are first set out so that, in a subsequent résumé of the experiments and observations which have led to our present knowledge, the aims and achievements of the individuals mentioned can automatically be relegated to their proper places in the general scheme of knowledge.

Shortly, it may be said that the main factor underlying an increased exudation of lymph is an increased permeability of the vascular endothelium. What the particular change may be that leads to this altered permeability is not yet sufficiently understood to render profitable an attempt to analyse it now, but the phrase itself carries a sufficient meaning to be useful.

Minor alterations in the degree of endothelial permeability are physiological necessities; an unending impermeability would be disastrous to the tissues around. Indeed, Rous, Gilding, and Smith have shown that different grades of permeability are present in different parts of the same healthy capillary vessel, the permeability of its endothelium being least at the arteriolar commencement of the vessel and greatest at the point where it terminates in a venule. Furthermore, slight differences in degree of endothelial permeation may follow such normal events as a mere change of posture. Thompson and Dailey, using the dye method for estimating the plasma volume of the blood, found that a change from the recumbent to the erect posture entailed a reduction of about 11 per cent. in plasma volume, the decrease being due to a loss of " approximately protein free fluid " which escaped from the bloodvessels of the legs. Waterfield has made somewhat similar observations. He used the carbon monoxide method and observed that the plasma volume was reduced by 15 per cent. when the recumbent was changed to the erect position, and accompanying this reduction was an increased concentration of protein in the plasma. This increase was of such a nature as would be expected if the globulin fraction alone did not escape through the capillary walls. Waterfield also

showed that coincidently with the reduction of the plasma volume was a swelling of the legs of such a degree as might be expected from the changes in the volume of the blood. These experimental findings illustrate minor changes of endothelial permeability and indicate also that there are various degrees of permeability, and that a capillary wall may be so altered that while it can be traversed by smaller particles to which ordinarily it is impermeable, such as serum albumen, it may remain impermeable to the larger particles of serum globulin.

Here a warning must be made concerning the use of misleading terminology. In discussing above the effects of postural changes these effects have been attributed to "slight differences of endothelial permeability." Such is the description which has come to be orthodox for want of some better definition. It is manifest that the permeability of a membrane is not to be measured by the amount and character of the substances passing through it in a given time, without a consideration of the forces which are responsible for the transport. The fallacy, though obvious, persistently recurs. The use of a special term, which is non-committal in the matter of causation, has been already suggested (p. 7).

In order that their resistance to excessive permeation may be preserved, the endothelial cells of the capillary vessels must be bathed by healthy, well-oxygenated blood, and any defect in such a provision will lead to an increase of their permeability to the circulating proteins. Among the chief causes of such an increased permeability are (1) a venous congestion of sufficient degree to cause stagnation with deoxygenation of the

blood and an accumulation therein of metabolites, (2) a stoppage of the arterial blood supply, (3) a defect in the relative amounts of the normal constituents of the blood itself apart from any circulatory disturbance, as occurs, for example, in nephritis, in which case the blood may be deficient in proteins, (4) the presence of active organic or inorganic poisons in the blood, of which histamine is an example. To these must be added (5) certain cellular degenerations affecting the endothelium—*e.g.*, amyloid disease, (6) inflammation, (7) imperfect recovery after injury, (8) immaturity, which may account for the increased permeability of the capillaries of young scar tissue, and perhaps (9) certain physiological conditions connected with functional activity.

As soon as an increase of endothelial permeability has become established from any of these causes, secondary factors, which at other times would have a negligible effect on exudation, will come into play and perhaps influence greatly the amount of lymph formation which ensues. Among these are active hyperæmia, increased hydrostatic pressure within the capillaries, currents of diffusion and osmosis, and differences of electrical potential. From these lists it will be seen that the various possible combinations of causative and secondary factors are too numerous for specific consideration under separate headings in the present chapter. Therefore it seems desirable to pass at once to a general review of the experimental work upon which our present knowledge is based.

Clinicians learned a long while ago to associate in certain cases the presence of a local œdema with blockage of the appropriate veins. Nevertheless in ordinary

conditions œdema is not an invariable nor, as a rule, a pronounced sequel of uncomplicated venous obstructions in man. Laboratory experiments upon animals too have led to differing results. The lack of uniformity between cause and effect in these instances is largely to be explained by the free collateral circulation in the venous system which is often sufficient to prevent stagnation of blood in the capillaries in spite of a closure by ligature or otherwise of the main channels for the return of the blood to the heart. Another source of fallacy in some laboratory experiments has been the fact that an interval of time must elapse for the stagnation to take much effect upon the capillary walls.

In former days the enhanced lymph flow following venous obstruction was attributed directly to the rise of hydrostatic pressure which ensued in the capillary tributaries of the obstructed vein; as a consequence of this misapprehension too little consideration was given to the necessity of allowing a sufficient lapse of time when performing experimental tests on man or animals. Today it is appreciated that the chief cause of an accelerated lymph flow following venous congestion is an excessive permeability of the capillary walls, brought about by a deficiency of oxygen and the prolonged contact of metabolites with the vascular endothelium. It is only when an increase of endothelial permeability has been established by such means that a rise of pressure within the capillaries can lead to a considerable increase of transudation.

Ranvier ligated the femoral vein of a dog immediately below the femoral ring and no œdema followed on the same or on succeeding days. He then ligated the inferior vena cava below the renal veins and again no

œdema resulted. Finally he divided the sciatic nerve on one side and a copious œdema ensued in the corresponding limb, but none could be observed in its fellow whose sciatic nerve was intact. He repeated this experiment on three different dogs with the same result on each occasion. He noted the following sequence of events. Soon after ligation of the inferior vena cava the hind limbs became cold; but after division of the sciatic the corresponding foot became warm and pink and the bloodvessels became full of blood, while the other foot remained pale and cold. Œdema was first noticeable one hour after the nerve section and was extensive at the end of twenty hours. Three days later the swelling began to diminish, and by the fifth day had disappeared. Another of Ranvier's experiments was as follows: the veins of both ears of a rabbit were ligated, and the cervical sympathetic nerve on one side was divided so as to cause vascular dilatation in the corresponding ear. Œdema now followed in the ear whose sympathetic nerve had been cut, but not in its fellow. From such data Ranvier was of opinion that the division of the vasomotor nerves and the consequent hyperæmia had been the chief factor in producing œdema. Commenting on this conclusion, Bouillard remarked that in man a complete and permanent interruption of the venous return was always followed by œdema of the part deprived of drainage, and he laid stress upon the varying degree of impediment to the circulation caused by obstruction of a vein.

Paschutin produced hyperæmia in the foreleg of a dog by dividing the brachial plexus and stimulating the spinal cord, and observed that no increase of lymph flow resulted. Other factors, he thought, than capillary

dilatation and increased intravascular pressure must come into play—perhaps an alteration in the capillary wall itself.

Emminghaus, following Ranvier's work, repeated his experiment, but reversed the procedure. Using dogs, he first divided the sciatic nerve and observed that this caused the corresponding paw to become hot, and yet no increase of lymph flow took place in the leg; he then caused venous obstruction and an increased exudation of lymph followed. He also found that irritation of the peripheral end of the divided sciatic nerve did not cause increased output of lymph.

Cohnheim appears to have had no doubt whatever that venous congestion alone could cause an increased exudation. He remarks that if one of the lymphatics on the outer side of a dog's leg be cannulated, so long as the animal is at rest but very little lymph escapes—scarcely a drop in several minutes. If, now, the principal veins are tied or the limb appropriately constricted, as many or more cubic centimetres of lymph will escape as did single drops before. Further, Cohnheim showed that the fluid exuded as a consequence of venous obstruction differed from normal lymph inasmuch as, while holding the same proportion of salts as plasma or normal lymph, it contained less protein than the latter, and had little tendency to clot.

This experiment illustrates a fallacy which Cohnheim avoided by analysing the "lymph." A mere increase of water and electrolytes escaping from the lymphatics in response to venous obstruction or other cause does not, strictly speaking, mean an increased flow of lymph, nor does it necessarily mean that the capillaries are unduly permeable. The true criteria in these matters

are the quantity and the kind of proteins which escape.

Accepting venous congestion as a cause of increased lymph flow, Cohnheim showed that the amount of fluid exuded might be greatly increased by adding to the passive congestion certain subsidiary factors. It may be mentioned incidentally that in certain acute cases of venous obstruction (strangulation) Cohnheim noticed a particular tendency for the exudation of lymph to be accompanied by a copious diapedesis of red cells.

Cohnheim, who agreed with Paschutin in the belief that an increased permeability of the capillary endothelium was the underlying factor of increased lymph formation, explained Ranvier's experiments in this way: he found that after ligation of the femoral vein in a dog the pressure within the vessel distal to the ligature rose to between 80 and 100 mm. of soda solution. If now the sciatic nerve were divided on the same side the venous pressure rose to 280 mm. in a few minutes, owing to the freer arterial influx into the limb. Such a rise of pressure, he thought, might well be considered as sufficient to explain the increased filtration of lymph through a damaged endothelium.

These observations upon the combined effect of venous obstruction and arteriolar dilatation in producing a more copious lymph flow have considerable importance in the field of therapeutics (p. 271).

Regarding subsidiary factors in lymph formation some joint experiments by Cohnheim and Lichtheim are of particular interest. Having tied the femoral vein of a dog and observed that no œdema of the paw

ensued, they produced an artificial hydræmic plethora by injecting a large volume of saline solution into one of the animal's veins. This additional procedure often, though not always, was followed by œdema of the foot in the limb whose veins had been occluded. In another experiment they maintained a simple hydræmia for a few days in the absence of plethora by withdrawing a certain amount of blood and replacing it by saline solution. In an animal so treated they found that ligation of a femoral vein was followed by œdema of the leg, while a considerable effusion of fluid occurred also in the tissues around the operation wound. The experimenters concluded that a continued hydræmia itself resulted in an injury of the vessel walls which thereby became more permeable.

Experimenting with rabbits and dogs Cohnheim and Lichtheim found that large intravenous injections of a sodium chloride solution did not produce general œdema, although they caused ascites and œdema of the alimentary canal. The experimenters noticed, however, that if at the time of the injection the animal had a healing wound, the tissues in the region of this wound became œdematous. They also found that a similar local œdema could be brought about by general hydræmic plethora in regions of the skin which had been painted with iodine or subjected to heat. Even sunburn was effective in eliciting the phenomenon. They shaved the abdomens of dogs and exposed them to the sun for one to two hours before an intravenous saline was given, and following the injection the areas of skin prepared in this way became œdematous. In other words, the only places where leakage occurred in easily recognisable quantity were (*a*) the abdomen,

(*b*) inflamed tissues, and (*c*) areas subjected to indirect injury through venous obstruction. Although Starling's work will be discussed more fully on another page, it may as well be stated here for the sake of clarity that he concluded from experimental evidence that the capillaries of the liver and intestines are normally more permeable or more readily rendered permeable than are the majority of the capillaries in the body, and therefore will at once respond to a rise of hydrostatic pressure by an increased rate of filtration, and to this fact may be attributed, perhaps, the ascites caused by artificial plethora (p. 32). But Cohnheim's observation that the vessels of the intestine, like those of the brain, are peculiarly subject to injury from depletion of their blood supply, may have a bearing on the point. On the other hand, the subcutaneous swelling which occurs in the areas of irritation and the œdema of the limb whose main vein has been tied are in each case the consequence of an increased permeability of the capillary endothelium resulting from direct trauma in the former and from indirect trauma, through obstruction of the circulation involving anoxæmia and accumulation of metabolites, in the latter.

In reference to the failure of cupping to produce an increase of exudation in the treated area, Cohnheim remarks that congestion by cupping differs *toto cœlo* from active hyperæmia because the partial vacuum within the cup exerts its action in all directions, so that blood will enter the region in question not merely from the arteries but also from the neighbouring capillaries and veins; and while in a hyperæmic tissue a large quantity of blood flows with increased velocity through the vessels, at the seat of cupping there is

a complete standstill. Lewis has offered a similar explanation for the failure of histamine to produce wheals when pricked into skin which is at once subjected to the influence of cupping. Some experiments by the author in connection with this subject are mentioned later (p. 70, and Plate V, B).

Cohnheim found that a temporary arrest of the blood flow caused by ligation or compression of the arterial supply was followed by increased exudation in the tissues to which the artery was distributed, and he further noted that the vessels of the brain and intestine were more easily damaged by arrest of their blood supply than were the vessels of the muscle and skin.

Reverting to the fact that, as a rule, observers have failed to find any increased exudation as a direct result of vasodilatation in the absence of a concomitant change in the capillary endothelium, an experiment of Rogowicz must be mentioned, as it is one exception to the rule. He divided the sciatic nerve of a dog and noted that a hyperæmia accompanied by a rise of temperature in the foot ensued. He now caused a rise of aortic pressure by stimulating the spinal cord. A slight temporary increase of lymph flow ensued in the paralysed limb; for example, in one experiment the volume of lymph flowing from the paralysed limb during the thirty-five minutes following the operation was 2·95 c.c., while the lymph from the normal limb was 1·0 c.c. The lymph was measured by catheterisation of a lymphatic vessel above the ankle.

Janowsky showed that nerve section in an inflamed limb brought about a pronounced increase of lymph flow. He anæsthetised a dog, and having caused venous obstruction by the application of a rubber band

to both hind limbs, the feet were dipped in water at 70° C. for about two minutes, at the end of which time epilation could be readily effected. The feet were then removed from the hot water and the constricting band was released. The right sciatic was then cut. At the end of half an hour both feet were inflamed and swollen. The lymph from both legs was collected, and to encourage its flow passive movements of the legs were carried out for five minutes at a time every ten minutes; 10 c.c. of lymph were collected in seventeen minutes from the limb whose sciatic nerve had been cut, while it took thirty-two minutes to collect the same quantity of lymph from the limb with intact nerves. The lymph did not differ in solid content on the two sides. On repeating this test a similar result was obtained. In another experiment Janowsky gave a subcutaneous injection of 5 grammes of turpentine emulsion into both hind feet of a dog. By the following day the feet had become inflamed. The animal was anæsthetised and the left sciatic was cut. As before, the rate of lymph flow was nearly doubled in the limb whose sciatic nerve had been divided.

The work of the investigators just mentioned reveals two facts worth considering. In the first place, it shows that arterial hyperæmia by itself does not cause a material increase of lymph formation, and secondly, it demonstrates that in the presence of an increased permeability of the capillary walls, an arterial hyperæmia plays an important part in augmenting the production of lymph. This adjuvant effect of hyperæmia on exudation recalls some analogous observations made by Barcroft and Müller on the part played by arterial dilatation in salivary secretion.

Heidenhain was unable to find any correlation between the aortic pressure and the rapidity of lymph flow from the thoracic duct in dogs. Ligation of the portal vein, which caused a slight fall of arterial pressure, increased the lymph flow more than four times. Moreover, this increased flow continued in spite of a fall of aortic pressure to zero, brought about by occlusion of the aorta. Intravenous injections of certain substances—leech extract, extract of crab muscle, peptone—caused an increase of lymph flow, while a similar injection of sugar reduced the output. From the results of his experiments Heidenhain concluded that the production of lymph was due to secretion rather than filtration, and he regarded the drugs which caused an increased output of lymph as lymphogogues. In the light of more recent knowledge their action is attributable to an increase of the permeability of the capillary walls. At that time the special permeability of the sinusoids of the liver was unknown, and the effects of anoxæmia and the accumulation of metabolites were not fully appreciated.

Lazarus Barlow carried out some experiments to elucidate the effect of venous congestion on lymph formation. He compressed the hind limb of a dog by a ligature which, though tight enough to obstruct the vein, did not obliterate the pulse in the leg. Lymph was collected by inserting a cannula into a lymphatic vessel running with the saphena vein. He found that an increase of venous pressure maintained for one hour was not accompanied by an increase in the amount of lymph flow, nor any œdema of the limb. A partial venous obstruction in man maintained for twenty-four hours produced a slight œdema limited to the proximity

of the ligature, and a similar effect was produced by the same means in dogs. If, however, complete hæmostasis by compression of the arteries were maintained for one hour prior to the venous obstruction, then the latter procedure was followed by an increased lymph flow and œdema of the leg ensued, but was first seen not in the proximity of the ligature, but in the distal part of the extremity. This experiment illustrates the readiness with which an increase of endothelial permeability may be produced by anoxæmia.

Starling's[1] work on the formation of lymph added so much to our knowledge of the escape of proteins from the blood, and especially from the sinusoids of the liver, that a summary of some of his more important findings will be given here. Using dogs anæsthetised with morphia and A.C.E. mixture, he found that:

(1) Obstruction of the inferior vena cava above the diaphragm caused the lymph flow from the thoracic duct to increase from 3 c.c. in ten minutes to 25 c.c. in the same space of time; and together with this increase in quantity was a rise in the percentage of solids from 4·8 per cent. before the obstruction to 6·6 per cent. afterwards. This experiment, he said, can always be repeated with success.

(2) Obstruction of the portal vein led to an increase of lymph flow from the thoracic duct, the lymph containing less than the normal amount of solids.

(3) Obstruction of the inferior vena cava above the diaphragm, together with ligation of the lymphatics which leave the liver at its hilum, was not followed by an increase of lymph flow from the thoracic duct, showing that the whole of the increase of lymph obtained after obstruction of the inferior vena cava

above the diaphragm was derived from the liver. This was true in spite of the fact that squeezing the liver in such conditions did not increase the flow of lymph, while squeezing the intestines did so. The reason was that lymph derived from the liver became collected in large lymphatic channels in the upper abdomen. A dye injected into the bile duct readily escaped into the lymphatics of the liver, and by utilising this fact, the connection between the hepatic lymph channels and those of the upper abdomen could be readily displayed.

Starling argued thus: blockage of the vena cava above the diaphragm causes a great rise of pressure in the vessel below the obstruction, and also in the portal vein, as shown by direct measurement, therefore it must produce a rise of capillary pressure in the liver while reducing the velocity of blood flow through this organ. Further, he found that occlusion of the aorta reduced the flow from the thoracic duct to one-third or two-thirds its former volume, though the percentage of solids was increased. Ligation of the aorta causes (*a*) a large fall of pressure in the arteries distal to the obstruction, (*b*) a moderate fall of pressure in the portal vein, (*c*) no fall or a slight rise in the inferior vena cava. Thus the pressure in the liver after occlusion of the aorta may be normal or slightly increased, though the amount of blood flowing through the organ in a given time will be diminished.

If, after the lymph flow has been reduced for thirty minutes by ligation of the aorta, the vena cava be now obstructed above the diaphragm, a large increase in lymph flow will at once ensue. Starling concludes that all the results of the procedures mentioned above point to the fact that an increased flow of lymph from

the liver is conditioned by increased capillary pressure. Liver lymph is more concentrated than other lymph. "The simplest way of explaining these differences is to look upon them as due to differences in the permeability of the filtering medium." The more permeable the medium the greater is the effect of changes of hydrostatic pressure of the filtering fluid. The capillaries of the limbs have only a small permeability, and the amount of transudation through them is but little affected by fairly large changes in the capillary blood pressure. The intestinal capillaries are more permeable. Highest in the scale of permeability come the capillaries of the liver. From these, there is in normal circumstances a flow of lymph, although the blood pressure in these vessels is very little above zero, but this lymph contains between 6 and 8 per cent. of proteins, and is therefore almost as concentrated as the plasma within the vessels, whereas the protein content of intestinal lymph varies from 4 to 6 per cent., and that of lymph from the extremities lies between 2 and 3 per cent.

Commenting upon Cohnheim and Lichtheim's experiments, in which they found that large intravenous injections of saline solution did not produce general œdema, although they caused a great increase of lymph flow from the thoracic duct together with ascites and œdema of the alimentary canal, Starling points out that hydræmic plethora is accompanied by a great rise of pressure in the portal vein and the vena cava, so that there is a large rise of hydrostatic pressure in the capillaries of the intestines and liver, which accounts for the ascites and the increased lymph flow from the thoracic duct. For if blood is withdrawn prior to the

injection of the saline solution, so that the latter merely dilutes the blood without adding to its volume, but little change is produced in the output of lymph.

In a later publication Starling[2] amplified his observations, noting in particular a difference in the readiness with which the bloodvessels of different organs might be rendered permeable. Thus, if all the veins of a dog's leg be ligated, an increased lymph flow may be set up, but such a flow is incomparably less than that produced by ligature of the portal vein. He explained the action of Heidenhain's lymphogogues as the result of an increased vascular permeability.

Starling noted that the permeability of the capillaries of a dog's leg can be altered by plunging the limb into water at 56° C. for some minutes, in which case lymph will begin to flow spontaneously from a cannula inserted into one of the crural lymph channels. This lymph is much richer in proteins than that derived from a normal leg. Moreover, the amount of lymph flowing from the leg can now be varied within limits by altering the pressure in the capillaries, either by ligature of the vein or artery, intravenous injection of salt solution, or the production of vasomotor paralysis. " By this scalding, in fact, we may reduce the limb capillaries to the condition of liver capillaries."

Bolton, following up the work of Cohnheim and Lichtheim and Starling, shed further light upon the matter of lymph production. Using cats, he found that obstruction of the vena cava above the diaphragm caused ascites and œdema of the retroperitoneal tissues, but no subcutaneous œdema, except where a wound had been inflicted. Cats were found to survive when the vena cava above the diaphragm, instead of being

completely occluded, had been narrowed so as to have a diameter of 2 mm. After an interval of time, the initial rise of pressure in the vein below such an obstruction subsided, but still the animals suffered from ascites. From this and from other considerations Bolton was able to affirm that the increased lymph flow which followed venous obstruction was attributable, not directly or alone to the rise of capillary pressure, but to a nutritional change in the walls of the capillaries leading to an increase of their permeability. Bolton and Barnard, in a recent paper, have confirmed these findings.

The idea that a variation in the permeability of the capillary endothelium might be an important factor in inflammation had occurred to Paschutin, and had been established as a fact by Cohnheim, who noted that exudation took place during inflammation in spite of a subnormal pressure in the arteries and capillaries of the inflamed part. The permeability of the vessel walls, Cohnheim concluded, is increased during, and owing to, inflammation, and it is for this reason that more liquid transudes through them in spite of the fall in capillary pressure.

The significance of such a variation of endothelial permeability as a physiological fact, and as an accompaniment of conditions other than inflammatory, became much more widely appreciated when Sherrington and Copeman published their observations on the transudation of plasma in general traumatic shock. These workers were inquiring into the effects of ligation of the splenic vein upon the specific gravity of the blood, and they were surprised to find that a considerable rise took place within a short while after the opera-

tion. Following up the matter, they discovered that every kind of operative trauma led to an increase of the specific gravity of the blood owing to a movement of the plasma from the vessels into the surrounding tissues. " Living cells," they say, " when put under stress beyond certain limits, instead of overriding and controlling the physical laws of filtration and osmosis, give way before them and react in obvious obedience to them." This leading observation has been supported by subsequent investigations. Reference will be made to a few of these.

Magnus caused hydræmic plethora in normal rabbits by means of saline solution without producing any subcutaneous œdema. Similar injections on dead animals, however, brought about extensive œdema, from which he concluded that the capillary walls of a healthy animal offer a resistance to the permeation of fluids, and that this resistance is lost when the animal dies.

Mann made a study of the blood volume in shock with the especial object of finding out what part, if any, was played by the medullary or vasomotor mechanism in bringing about the loss of fluid. After various operative procedures he recorded the percentage of the estimated total blood which could be recovered by bleeding the animal first from the femoral artery and then from the right auricle. His results are given in the appended table, which shows that the lowest proportion of the estimated total of blood was recovered from the animals which had been subjected to severe trauma, thus confirming by a direct method the loss of blood volume in traumatic shock.

PERCENTAGES OF TOTAL BLOOD OBTAINED.

	From Femoral Artery.	*From Right Auricle.*	*Total.*
Normal dog	66	10	76
Dog with cervical cord cut to produce medullary vasomotor paralysis.	54	12	66
Dog with blood pressure reduced almost to zero by overdose of ether.	46	13	59
Dog reduced to state of traumatic shock by exposure of viscera.	28	11	39

A great advance followed the researches of Dale and his co-workers into the action of histamine and some other substances which affect the calibre of the small bloodvessels. Dale and Richards observed that the vasodilator effect of histamine upon the capillaries was independent of the integrity of any nervous connection, and they suggested that substances with a histamine-like action were produced by activity or injury of the tissues. In a paper which followed shortly after the one to which reference has just been made, Dale and Laidlaw enlarged further on the action of histamine, showing that it caused relaxation of the capillaries together with an increased permeability of their walls, which allowed the escape of plasma from the blood. Local injections into or beneath the skin caused redness and œdema. Given intravenously to cats, the drug caused symptoms resembling those of traumatic shock, that is to say, a lowering of the arterial pressure in spite of constriction of the arteries, an increased content in the blood of red cells owing to a loss of plasma,

a fall in the cardiac output, and a reduction of the volume of the blood. They added that other substances than histamine caused similar symptoms, and they were the first to point out the similarity of the results arising from intravenous injections of histamine and certain other poisons into normal animals on the one hand, and of antigen into a sensitised animal on the other, among these results being an oligæmia due to the escape of plasma from the bloodvessels. The importance of these observations of Dale and his fellow-workers in elucidating the mechanism of shock and inflammation is great, and forms a foundation for all subsequent investigations of the problem.

Petersen and Levinson were able to demonstrate an increase of endothelial permeability during general anaphylactic shock in the following manner. Dogs were sensitised by giving egg-white to them intravenously on three successive days. Two weeks later a thoracic duct fistula was made, under local anæsthesia, and the lymph collected. A moderate degree of anaphylactic shock was now brought about by an intravenous injection of 2 c.c. of a 20 per cent. solution of egg-white. An increase of lymph flow resulted almost at once, the volume rising from 1·35 c.c. in fifteen minutes to 11·5 c.c. in a like period. Moreover, the lymph of shock was richer than normal lymph in fibrin and globulin.

When severe anaphylaxis was caused so that the animal died two hours after the injection, the concentration of protein in the lymph actually exceeded that in the same amount of plasma collected at the same time.

Manwaring, Chilcote and Hosepian investigated the

local anaphylactic reaction in the lungs of dogs. With the trachea clamped, the pulmonary vessels were perfused with Locke's solution containing 1 per cent. of horse serum. When the lungs of normal, unsensitised dogs were used the perfusion rate remained steady, the outflow being equal to the inflow, and when, at the end of the perfusion, the tracheal clamp was released, the lungs collapsed normally and no frothy fluid escaped from the trachea. On the other hand, if they used the lungs of a dog which had previously been made sensitive to horse serum the consequences were quite different, for the perfusion flow dropped rapidly from 1,500 c.c. per minute to 300 c.c. per minute, the lungs increased in size, became of a rubber-like consistency, and did not collapse when the tracheal clamp was released, while a large amount of frothy fluid escaped from the trachea. If the perfusion was still continued after this, fluid poured out of the trachea almost as rapidly as it escaped from the efferent cannula. From this the experimenters concluded that increased endothelial permeability was the dominant change in protein sensitisation and anaphylactic shock.

The most permeable part of the small vessels appears to be about the point where the capillaries terminate in venules. Tannenberg has observed that in the mesentery of a rabbit diapedesis of leucocytes commences in the smallest veins, and follows then in the venous ends of the capillaries and comes about lastly in the arteriolar ends of the capillaries and in the arterioles.

Rous, Gilding and Smith gave injections of Chicago blue into rabbits and guinea-pigs and then watched its

escape through the capillaries of the external oblique muscle of the abdomen. Although reaching the arteriolar ends of the capillaries first, where the hydrostatic pressure was highest and the dye most concentrated, permeation began at the venous ends of the capillaries—that is to say, where the oxygen tension was lowest. It appeared that the permeability of the capillaries increased progressively along their course from arterioles to veins. This characteristic gradient of permeability was not disturbed by such drastic circulatory changes as plethora on the one hand or exsanguination on the other.

So far these experiments appeared to support the view that oxygen tension and permeability were in some way closely related. In subsequent papers, however, Rous and Smith have shown that a similar gradient of vascular permeability exists in the frog not only in the muscles, but in the skin covering the lower abdomen. And owing to the peculiar arrangement of the frog's bloodvessels in this region which subserve the function of cutaneous respiration, the oxygen tension is said to be higher in the blood which leaves the area than in the blood which enters it. Accepting this, it is necessary to admit that in the frog's skin there are factors more influential than anoxæmia in regulating the permeability of the walls of the small vessels. The interesting observations of Rous and Smith on the frog cannot be made to bear on the problem as met with in man without great reserve. Not only are the respiratory functions in man and the frog widely different, but the capillaries of the frog, and particularly those of the skin, differ from human capillaries inasmuch as the former are freely permeable

to proteins in normal circumstances, as both Conklin and Drinker have shown.

Landis,[4] as the result of direct observations of the capillary circulation in the frog's mesentery, states that complete saturation with CO_2 had but little effect, and an increase of hydrogen ion concentration within physiological limits had no effect upon the permeability of the capillary endothelium; a three-minute oxygen lack, on the other hand, caused fluid to filter through the capillary wall four times as fast as usual. His experiments with trypan red seemed to show that the capillary of the frog's mesentery was most freely permeable towards the arteriolar end. Rous and Smith have re-examined the matter, using several dyes—trypan red, brom-phenol blue, trypan blue, and Chicago blue 6B—and their findings have been consistent. The dyes regularly escaped first from the distal portions of the capillaries and the smallest venules. Often none was seen to get out anywhere else. They remark that a relatively abundant escape from the arteriolar region of the capillaries was always traceable either to great diffusibility of the dye or to a damaged capillary system; and their explanation of Landis's contrary results is to the effect that his observations were made upon injured vessels.

That lymph varies in constitution in conformity with the circumstances in which it is produced was noted by Cohnheim, who observed that in conditions of hydræmia inflammatory lymph contained less protein than was present in exudates formed when the protein content of the blood was normal. He further noted that the lymph of venous congestion contained less protein than was present in an inflammatory

exudate, while the latter formed a more substantial clot.

To some extent the total amount of solids in the lymph, or merely the quantity of proteins, may be taken—other things being equal—as an index of endothelial permeability. Not only so, but the relative proportion in which the different proteins are present supplies a similar indication, for the molecules of fibrinogen, which presumably are larger, pass less easily and rapidly through the endothelium than do the smaller molecules of serum albumen.

Unna states that the albuminous exudate produced by applying cantharidin to the skin is at first almost free from fibrinogen; and in this connection it may be noted that Kulisch, working in Unna's laboratory, found that cantharidin, though bringing about vesicles, caused hardly any reddening of the skin. It may be that the absence of vaso-dilatation in inflammation caused by cantharidin is responsible for the nature of the exudate which results.

The selective character of exudation led Cohnheim to adopt the idea previously suggested by Paschutin that it depended upon a molecular permeability of the capillary wall. Had the process been contingent on the presence of stomata, as had been proposed to explain the occurrence of diapedesis, an inflammatory exudate would have consisted of unaltered plasma, which is not the case.

Lewis[2] was able to analyse the fluid of wheals which had been produced by stroking the skin in patients with factitious urticaria, and he found that the protein content of the wheal fluid more closely approached that of the blood serum than does that of lymph collected

from the limb or the fluid drained from dropsical patients—a fact which forms further evidence, as Lewis remarks, of an increased vascular permeability during the formation of the wheal.

Summary

The transudation of proteins is rendered possible by an increased permeability of the vascular endothelium. Among the causes of such an increase are the following:

(1) Venous congestion (anoxæmia and accumulation of metabolites).

(2) Anoxæmia.

(3) Defective quality of blood (hydræmia).

(4) Presence of organic or inorganic poisons in bloodstream (histamine, Heidenhain's lymphogogues).

(5) Degeneration of endothelium (amyloid).

(6) Inflammation.

(7) Imperfect recovery after injury.

(8) Immaturity of endothelium.

(9) Possibly certain physiological conditions associated with functional activities.

In the presence of increased vascular permeability a number of secondary factors come into play and greatly influence the rate of transudation. Among these secondary factors are (1) hyperæmia, (2) an increased hydrostatic pressure within the capillaries—whether due to arterial dilatation, venous compression or to both combined, (3) osmotic pressure, (4) diffusion currents, and (5) electrophoresis.

CHAPTER IV

THE LOCALISATION OF FOREIGN PROTEINS

SINCE the normal plasma proteins escape through the capillary walls in inflammation and in certain other circumstances mentioned in the previous chapter, it may be assumed that foreign proteins will follow the same course. Relatively little experimental work has been done in this field, but so far as it goes it justifies the assumption. Not only do foreign proteins thus gain entrance to the extravascular regions, but under the influence of inflammation, they become retained there. In other words, if a foreign protein be introduced into the bloodstream it will become concentrated in any focus of inflammation that is present.

Auer, while testing the sensitiveness of dogs which had been treated with horse serum some years before, and using heavy intravenous doses of horse serum for these re-injections, noticed that an œdema ensued around the incisions which had been made recently in the groin. It occurred to him that this unusual œdema might be of anaphylactic origin; that is to say, with the ordinary reactive inflammation in the wound there would be an exudation of plasma carrying with it some of the injected horse serum which, brought thus into direct contact with sensitised tissues, would result in a local anaphylactic reaction. To test this hypothesis he carried out a series of experiments on rabbits which he sensitised by several injections of 4 c.c. of horse serum given at four- or five-day intervals, half the

injections being made into the peritoneal cavity and half into the erector spinæ muscles. From fifteen to twenty days later the rabbits were given 10 c.c. of horse serum intraperitoneally, and half an hour or three-quarters of an hour later 1 c.c. of xylol was rubbed into one ear of each rabbit. The rubbing was done gently with a finger covered with a rubber cot, and was continued for fifteen or thirty seconds. As a consequence of this treatment a severe inflammation, sometimes followed by gangrene, was brought about in the ear to which xylol had been applied. Xylol applied in the same way to normal rabbits, or to rabbits which had been sensitised to horse serum but had not been reinjected, caused merely a slight inflammation with œdema which disappeared in two or three days, leaving a normal ear.

The results of this experiment are readily explained if we suppose that the inflammation set up in the ear by the application of xylol has led to an increase of the permeability of the capillary walls in this region with a local accumulation of foreign protein in the tissues as a consequence.

Opie has repeated Auer's experiment on rabbits and confirmed his results, using crystalline egg albumen as the antigen and inflaming the ears by dipping them for five minutes into water at a temperature of 50° C. Necrosis of the inflamed ear was observed in many instances. He[1] further demonstrated the retention of foreign proteins in tissues under the influence of antigen-antibody reactions. Thus horse serum or egg white injected into the subcutaneous tissue of a normal rabbit almost at once enters the bloodstream, where it can be detected for seven or eight days afterwards,

its disappearance following shortly after the appearance of precipitin. With repeated injections of antigen there arises in the animal so treated an increasing opposition against its access to the bloodstream, so that, in an animal well-immunised against horse serum or egg albumen, these substances, after subcutaneous injection in moderate quantity, cannot be detected in the blood even if repeated tests are made at intervals of from one to twenty-four hours after the injection; though by injecting massive doses the capacity of the immunised animal to prevent the antigen from entering the blood may be overcome. Opie[2, 3] believes that wherever antigen and antibody (precipitin) meet within the body an inflammation occurs—this being the basis of the Arthus phenomenon and of several of the specific skin tests used in clinical practice. Opie failed to desensitise rabbits to horse serum by injecting it in massive doses, nor in these circumstances did precipitin disappear from the blood; but using crystalline egg albumen in large doses, precipitin disappeared from the blood and complete desensitisation of the tissues was effected. Opie and Furth, by the production of reversed anaphylaxis, showed that it is unnecessary to assume any essential alteration of the tissues themselves as the consequence of sensitisation. To a normal rabbit is given 10 c.c. of horse serum in the subcutaneous tissue or an ear vein. On the next day 0·5 c.c. of strong anti-horse-rabbit serum is injected into the skin and produces extensive œdema at the site of injection, whereas an injection of normal rabbit serum, the conditions otherwise being the same, does not produce any such inflammatory reaction.

Opie[4] made estimations of the concentration of

foreign proteins in the fluids of tissues, following the introduction of these antigens subcutaneously into normal and immunised animals, by excising the tissues, extracting them with saline solution, and testing the extract by the precipitin reaction. Using crystalline egg albumen in this way, he found the concentration at the site of injection to be 1 : 250 on the first day and 1 : 500 on the second day in the case of a normal animal, whereas in a sensitised animal the ratios were 1 : 20 and 1 : 50 respectively. When 0·5 c.c. of horse serum was injected into each of two normal rabbits, 0·17 c.c. and 0·284 c.c. were recovered from the treated tissues on the following day, whereas when similar quantities were injected into two immunised animals, 0·46 c.c. and 0·449 c.c. were obtained on the next day. Opie believes that specific precipitation may play a part in this fixation of foreign proteins in tissues which are subjected to anaphylactic inflammation.

A comparable phenomenon had already been observed by Rous, Wilson, and Oliver while inquiring into the possibility of producing antisera against unidentified infections in man. They found that the serum of rabbits which had been given injections of serum from guinea-pigs or dogs was highly toxic for the animals which had furnished the antigen. Given to these animals intravenously it caused sudden death, and injected subcutaneously it produced acute inflammation. The complete removal of hæmolysins and hæmagglutinins by exposing the serum repeatedly to washed red cells lessened its toxicity but little, and removal of precipitin by specific precipitation *in vitro* had no detoxicating effect. The symptoms produced resembled anaphylaxis, but attempts at desensitisation

failed. Whatever the reaction may depend upon, the inflammation produced will tend to bring about a retention of any foreign proteins at the site of their injection.

Menkin[3] has repeated and confirmed Auer's experimental demonstration, and has brought forward even more convincing evidence of the attraction exercised by inflamed tissue for circulating antigens. Areas of cutaneous inflammation were induced in rabbits by the injection of about 0·2 c.c. of a saline suspension of *Staphylococcus aureus* into the skin of the abdomen. Three hours later 10 c.c. of horse serum were given intravenously. The animals were killed a few hours later. The inflamed areas were excised, sliced, and ground in a mortar with twice their amount of saline solution. Strips of normal skin were removed from the abdomen of the same rabbit and treated in the same way. The extracts of the inflamed and the normal skin were then tested by means of anti-horse serum obtained from rabbits which had been repeatedly injected with horse serum. There was shown to be a consistently greater concentration of the foreign protein in the inflamed than in the uninflamed tissue.

In another form of experiment Menkin sensitised a rabbit by repeated injections of horse serum, and nineteen days after the last injection he caused inflammation in one ear by holding it in water at 54° C. for five minutes; 10 c.c. of horse serum were now injected into the peritoneum. Twenty-four hours later the rabbit was bled to death under ether, and the ears were removed, weighed, and separately sliced and ground up in equivalent amounts of saline. Precipitin tests carried out on the extracts prepared

from them showed their relative contents of foreign protein to be as shown in the appended table:

Dilutions of Skin Extract.	*Normal Ear.*	*Inflamed Ear.*
1 : 3	+ +	+ + +
1 : 9	Trace.	+ +
1 : 27	0	+
1 : 81	0	+
1 : 243	0	0

From this it will be seen that antigen was collected in greater concentration in the inflamed than in the normal ear.

Menkin further observed that horse serum, introduced into a rabbit's peritoneum which had been inflamed, entered the bloodstream more slowly than when introduced into the normal peritoneum. His experiment was as follows. Aseptic peritonitis was induced by an injection of aleuronat and starch. Between twenty-four and forty-eight hours later 2 or 4 c.c. of horse serum were injected into the peritoneum. Each experiment was controlled by the introduction of an equal amount of horse serum into the peritoneum of a normal rabbit. From two to five hours later 10 c.c. of normal saline solution were injected into each peritoneum. The rabbits were then bled from the heart and peritoneal fluid was withdrawn, the animal having been anæsthetised with ether. Both blood and peritoneal fluid were tested by means of anti-horse serum obtained from rabbits which had received repeated injections of horse serum. By these means Menkin found a greater quantity of horse serum in the blood and less in the peritoneum in the normal rabbits than was present in the animals in whom aseptic peritonitis had been induced.

Some interesting experiments carried out in connection with the problem of sensitivity appear to illustrate Opie's dictum as to the mutual fixation of antibody and antigen through the medium of inflammation.

Brunner and Walzer caused local sensitivity to fish in a number of normal individuals by administering intradermal injections of 0·05 c.c. of a serum obtained from patients known to be fish-sensitive. Next day each experimental individual was given on a fasting stomach one-third of a raw herring. Within a relatively short period, varying from a few minutes to two hours, this usually produced pruritus, erythema, and a wheal at the sensitised site. "This reaction could only be effected by the union of the reagins," and it therefore showed that unaltered fish protein had entered the circulation from the digestive tract. This reaction occurred in 93·8 per cent. of sixty-five persons tested. Fish-sensitive patients, on the other hand, showed a diminished reaction or none at all. The presumption is that in the latter cases, owing to the pre-existence of anti-fish substances in the tissues, the fish proteins were fixed at once and so hindered or prevented altogether from gaining access to the general circulation.

A somewhat analogous experiment was carried out by Cohen, Ecker, Breithart, and Rudolph, also based upon the Prausnitz-Küstner skin reaction. This experiment was as follows: about 0·2 c.c. of blood plasma obtained from a patient known to be sensitive to "short ragweed extract" was injected into the skin of a normal person, and into another area of skin was injected 0·2 c.c. of normal plasma. Twenty-four hours later pollen was insufflated into the individual's nostrils. In twenty-five normal people so treated a wheal

began to appear in the sensitised area of skin within half an hour, but no wheal occurred in the skin treated with normal plasma. In addition to these normal individuals the test was carried out on twelve others who were known to be atopic; that is to say, they had suffered from asthma, hay fever, or vasomotor rhinitis, but were not sensitive to ragweed; nine of them had given definite skin reactions to one or more test substances used in routine work, the remaining three having been negative to these tests. The results of the pollen test on these atopic individuals was as follows: The three who had proved negative to previous tests gave normal skin reactions, that is to say, a local response in the sensitised skin within half an hour of applying pollen to the nasal mucosa, whereas the remaining nine all showed a pronounced delay in the reaction or no reaction at all. The conclusion suggested is that in the sensitive subjects there is some local mechanism whereby absorption of the antigen by the mucous membrane of the nose or its distribution therefrom is delayed or entirely prohibited.

The lack of specificity in this test need not concern us, for it will be made clear in subsequent pages that any inflammatory condition in a tissue, regardless of its cause, may be expected to prevent or delay the diffusion therefrom of an antigen.

Other examples of the localisation by inflammation of foreign proteins have been reported in connection with fixation abscesses. Netter and Césari,[1] having treated successfully a case of pneumococcal meningitis by means of a sterile fixation abscess in the thigh produced by turpentine, found that antigens of the infecting organism were present in the pus. For this test they

used Ascoli's method: that is to say, equal weights of distilled water and of pus from the thigh were mixed and kept in a refrigerator for twenty-four hours, then filtered and made isotonic by the addition of a 20 per cent. solution of sodium chloride at the rate of one drop per cubic centimetre of filtrate. One cubic centimetre of various agglutinating sera was placed in each of several test-tubes and an equal quantity of the filtrate from the abscess poured gently down the side of each test-tube. When a filtrate containing antigen is added in this way to a serum which carries the corresponding antibody, a pronounced precipitate appears at the end of some hours where the two liquids meet. In the case under review, such a precipitate was obtained by adding the abscess filtrate to an antipneumococcus Type II. horse serum. Subsequently Netter and Césari[2] have confirmed this primary observation by means of additional cases.

Though perhaps not properly included in a discussion confined to the localisation of foreign proteins, it will be convenient here to recall an experiment of Belin, who discovered that the pus from a fixation abscess, provoked twenty days after the last injection of tetanus toxin into a hyperimmunised horse, was very rich in antitoxin.

Clearly more experimental work is required in this field. Meanwhile, what evidence we have suggests not only that foreign proteins pass out from the capillaries as well as plasma when inflammation is present, but that they are retained and become concentrated in the inflamed zone.

CHAPTER V

THE LOCALISATION OF DYES AND FINE INORGANIC PARTICLES

It is well known that when colouring matters in colloidal form, and carrying a negative electrical charge, are injected into the bloodstream, they are taken therefrom by the sinusoidal endothelium of the reticulo-endothelial organs, particularly the liver, bone-marrow, and spleen. When a focus of inflammation is present, an alternative destination is provided. The walls of the blood capillaries of an inflamed region are pervious to colloids, and indeed to substances whose particles are of a larger size. These substances, under influences which are not yet completely defined, will leave the bloodvessels, and entering the inflamed tissues will be retained by them. Here, to a large extent, they will be ingested by the tissue macrophages. This localisation of colloidal and other matters in the reticulo-endothelial organs and in inflamed tissues seems to be but little influenced by the chemical constitution and special affinities of the matters themselves, and to depend almost entirely on the nature of the electrical charge which they carry and on the size of the particles.

From experimental work on the living animal it appears that only matters which are negatively charged when in the blood become localised by inflammation. This subject will be discussed with greater detail in a subsequent chapter. Meanwhile, it is to be understood that most, if not all, the substances mentioned

below as becoming deposited in zones of irritation are known to carry negative electrical charges when dissolved or suspended in water.

Most of the experimental work upon the destination of foreign substances in the animal organism has been carried out with dyes and other coloured matters, not because these—speaking broadly—have a distribution peculiar to themselves, but because their fate can be readily detected with the eye alone. The earliest recorded observations on vital staining appear to have been those of Belchier which were made nearly two hundred years ago, but for the purposes of the present work it is not necessary to revert to any experiments earlier than those of Hoffmann and von Recklinghausen, the details of which were published in 1867, immediately after, and in response to, Cohnheim's paper on "Inflammation and Pus Formation."

Hoffmann and von Recklinghausen seem to have been the first to observe the fixation of colouring matter in an inflamed organ. While enquiring into the origin of pus corpuscles they injected cinnabar into the dorsal lymph sacs of frogs, and either immediately or a few days later, caused keratitis by applying lunar caustic to the eye. This was followed by the appearance of a red zone of cinnabar which, appearing at the margin, gradually spread toward the centre of the cornea. The granules of cinnabar were segregated in the pus corpuscles, which appeared in large numbers. Whether any of the particles were free from enclosure within corpuscles they were unable to decide. These experimenters noted also that cinnabar became lodged in certain of the internal organs, notably the liver, spleen, and kidneys. Microscopically the granules appeared

in the greatest number "in those contractile cells of the liver and spleen which simultaneously contain masses of pigment and red blood corpuscles." It is clear by these and some further experiments which will be mentioned later (p. 241) that Hoffmann and von Recklinghausen were already outlining the macrophage system, a fact which is worth noting in view of the early date of their publication.

Eighteen years later Rogowicz cut the cervical sympathetic nerve of a rabbit and then injected into the saphenous vein a 1 per cent. solution of indigo sodium sulphate until the normal ear showed the first sign of coloration, by which time the other ear was stained a pronounced blue. In another experiment he stimulated one lingual nerve of a dog which was under the influence of morphia. While this was going on he injected indigo sodium sulphate into the saphenous vein, and found that the half of the tongue corresponding to the stimulated nerve became deeply blue while the other half showed only a trace of colour. As the operation proceeded, however, in both of these instances the coloration of the two sides tended to become equal. In another series of experiments Rogowicz cut the sciatic nerve of one leg in a dog. Then, having catheterised the lymphatic vessels of each leg, he injected indigo sodium sulphate into the animal's anterior facial vein. In five such experiments he found in all that the lymph derived from the limb on which nerve section had been performed became blue earlier than that from the normal limb and continued to be more deeply stained. He had already observed that section of the sciatic nerve led to a small temporary increase of lymph flow from that limb (p. 29).

In these experiments we have to remember that, with a dye present in the circulating blood, a region in which the vessels are dilated will appear more highly coloured than will a normal tissue, even when none of the dye has escaped from the bloodvessels. Nevertheless, Rogowicz certainly seems to have shown that the hyperæmia produced by nerve section and stimulation is accompanied by a temporary increase of lymph formation, and that a dye present in the blood may pass in these circumstances into the lymph and so drain away through the lymphatic channels. In other words, diapiresis of the dye occurs, but in the absence of local inflammation there is no retention of the dye in the tissues to which it has become distributed.

Dreser, in 1887, used fuchsin to show the formation of acid in active muscle. The dye was introduced as a 5 per cent. solution into the dorsal lymph sac of a frog. Muscles at rest showed hardly any coloration owing to their alkalinity—for the red colour of the dye is abolished by alkali. If, however, the circulation of one of the hind limbs was stopped by ligation while the sciatic nerve was irritated so as to tetanise the muscles, an obvious reddening of the limb occurred. There is no necessity to discuss Dreser's experiments at further length, as they are germane to the present work only from the general historical standpoint, and because they led by chance to a remarkable series of observations bearing upon the influence of injury on the permeability of the bloodvessels of the brain (p. 59).

Goldmann, whose work was first published in 1909, made a material addition to our knowledge of the

localisation of dyes from the bloodstream. Goldmann, using white mice, gave repeated subcutaneous injections of isamine blue until the animal had become generally stained. In his very first experiment he noticed that a lesion of the ear, resulting from a slight cauterisation, induced the blue colour to appear there before it could be seen anywhere else. When animals were injected in a very early stage of pregnancy, the nipples showed up as dark points on the bright background of the surrounding abdominal skin, and if no further dye was given the general skin became paler and paler until finally the nipples alone remained darkly coloured in the "de-blued" skin. On killing a pregnant animal stained during life with isamine blue he found that the placenta, the fœtal membranes and the amniotic fluid were blue, while the fœtus remained quite uncoloured. In the mamma, during lactation, he found an extraordinary increase in the number of macrophages which were loaded with the dye ("pyrrhol cells").

Since Goldmann's first paper appeared a succession of experimenters have interested themselves in the subject from various standpoints.

Bowman, Winternitz, and Evans used injections of dyes in order to show up early lesions in experimental tuberculosis in animals, while Winternitz and Hirschfelder observed that trypan blue or trypan red, when given intravenously to rabbits with experimental lobar pneumonia, became selectively localised in the consolidated parts of the lungs.

Tschaschin, studying the macrophages, produced lesions of the mesenteric lymph glands, liver, and spleen by piercing them with a red-hot needle, and introduced

PLATE II

Localisation of dye in the placenta.

Isamine blue has been given intravenously to a pregnant rat. The dye has become localised in the maternal portions of the placentae.

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isamine blue and other substances into the bloodstream of the animals during the period of repair. He subsequently noticed that the phagocytes collected around the lesions had taken up large amounts of the dyes. He also caused aseptic inflammation of the subcutaneous and intermuscular connective tissues by introducing celloidin foreign bodies, and then gave intravenous injections of isamine blue, and again he found the assembled macrophages to be deeply stained.

P. A. Lewis caused conjunctivitis in rabbits by inoculating the cornea with a living culture of tubercle bacilli, and twenty-four hours later he gave an intravenous injection of trypan red. In other experiments he produced conjunctivitis by applications of abrin, accompanied by intravenous injections of trypan red or trypan blue, and he found that these dyes became localised in the conjunctiva, cornea and anterior chamber in both sets of experiments.

Cecil used injections of trypan blue to stain the macrophages in local inflammatory lesions artificially produced in rabbits by inoculating them with streptococci, and Macklin gave intraperitoneal doses of trypan blue in order to stain the macrophages around broken bones, and to study their activities during repair.

An interesting series of experiments carried out by Abel and his colleagues, and connected with the action of convulsant drugs on frogs, may be mentioned here. Acid fuchsin had been used by Dreser to demonstrate the liberation of acid in active muscle, and it was found that the dye caused convulsions in frogs if injected into the dorsal lymph sac in sufficient quantity. Barbour and Abel corroborated a previous observation made by Abel[1, 2] to the effect that these convulsions were pro-

duced in a shorter time and by much smaller doses of acid fuchsin if the anterior part of the forebrain had been removed. They found, also, that smaller doses were required to produce convulsions in frogs which had become exhausted from physical exertion than in normal frogs. The first explanation suggested for these phenomena was that in the healthy frog the convulsions were hindered by inhibitory impulses derived from the forebrain. This explanation later had to give place to one which is in full accord with the theme of this essay—namely, that injury of the brain is followed by an increased permeability of the capillaries of the injured part, by which means the acid fuchsin has a more ready access to the nervous tissue concerned.

Curiously enough, it was found by Joseph and Meltzer that smaller doses of convulsant dyes are effective, after their injection into the dorsal lymph sac, in frogs which have been submitted to cardiectomy than in frogs with a normal circulation. Nevertheless, Abel succeeded in showing that all frogs which had convulsions after injections of acid fuchsin, whether after cardiectomy or cerebral injury, had this in common—namely, a visible concentration of acid fuchsin in the brain; and a little later Abel and Turner were able to explain the surprising phenomenon noted by Joseph and Meltzer by showing that the anterior lymph hearts are in direct communication with the vertebral veins into which they pump the lymph from the dorsal lymph sac; wherefore a dye when introduced into the dorsal lymph sac will reach the central nervous system in a higher concentration in frogs which have been submitted to cardiectomy than in normal animals. After cardiectomy together with destruction of the

lymph hearts by cauterisation, the administration of fuchsin no longer was followed by convulsions, owing to the fact that the dye was not conveyed to the brain.

Macht followed up this work and was able to report similar convulsant effects on frogs in the same circumstances from several substances, including phenol-sulphone-phthalein, naphthol yellow, tropæolin-oo, Basel I and Basel III, all of which, like acid fuchsin, are water-soluble sulphonated dyes.

Thomas found that a simple pin-prick in the region of the optic lobes or immediately anterior to them was just as effective as decerebration in facilitating convulsions after the administration of acid fuchsin to frogs. Moreover, with limited injuries of the spinal cord inflicted prior to giving the dye, he was able to produce spasms confined to the muscles supplied by the segments of the cord concerned with the injury. Syz, continuing this inquiry at Professor Abel's suggestion, confirmed the observations of Thomas, finding that cerebral injuries caused localisation of the dye in and about the injured tissues, and that such localisation of the dye led to convulsions. If, after an injection of fuchsin had been made into the dorsal lymph sac, the brain of a living frog was exposed without damaging it, no pink coloration appeared in the brain, nor was the animal convulsed. If, however, the brain was pricked, it became pink and convulsions occurred. Applying the dye directly to the uninjured surface of the brain did not have any convulsive effect.

In connection with these experiments it is interesting to recall an observation made by Sauerbruch to the effect that monkeys and rabbits are much more susceptible to cocaine after the motor cortex of the left

cerebral hemisphere has been injured, when convulsions appear after the subcutaneous injection of a dose one-fifth of that required to bring about the same effect in an uninjured animal. Fatigue also allows smaller doses of cocaine to cause convulsions. Although Sauerbruch did not put such an interpretation upon them, we are justified in suspecting that these consequences may have been due to an increased permeability of the cerebral vessels. They appear to the writer to be the counterpart of the experimental results obtained by Abel and his colleagues with convulsant drugs administered to frogs.

Macklin and Macklin injected trypan blue intraperitoneally into rats, and then gave them blows on the head, and they found that this trauma was sufficient to localise the dye in the brain. A similar staining of the brain and spinal cord of rats was produced by trypan blue in the presence of experimental meningitis. McClellan and Goodpasture used injections of trypan blue in order to identify lesions of the central nervous system brought about in rabbits by the virus of herpes febrilis. They found the method of great value. MacCurdy and Evans had already used this method in monkeys. They remark that while normal brain and spinal cord always remain free from dye injected intravenously, areas of damage—softening or inflammation—become deeply stained.

Siengalewicz gave intravenous injections of trypan blue to rabbits, which were then placed in a box in which they inhaled coal gas in such concentration that signs of poisoning occurred within from thirty to sixty minutes. The animals were then killed, and it was found in a total of eleven experiments that the whole

brain—cortex, basal ganglia, and white matter—were always stained blue, in contrast with normal animals whose central nervous system does not become stained after injections of this dye given during life. It has been noticed by several pathologists that extensive areas of cerebral softening are found in human beings who have died as the result of carbon monoxide poisoning after surviving long enough for these changes to take place. On this matter reference may be made to an informative paper by Semerak and Bacon. Siengalewicz observed that poisoning by salvarsan also led to an abnormal colouring of the brain by trypan blue, but in this case the staining was limited to the neighbourhood of the ventricles.

Russell gave intraperitoneal injections of trypan blue to a rabbit, and three hours later tapped a fine nail into the animal's brain. She observed that the microglia cells of the brain around the wound gradually lost their branching processes, assumed a rounded form, and became extremely phagocytic, taking up large quantities of the dye which had localised in the inflamed region.

Ebbecke has reported that if trypan blue or trypan red be injected into the general bloodstream and a wheal be then produced in the skin, the dye will discolour the wheal, but if the wheal be brought about prior to the administration of the dye, no discoloration of the wheal will occur.

Experimenting with paraphenylenediamine, which causes a specific œdema of the head and neck in rabbits, Tainter and Hanzlik noticed that congo red given intravenously stained the œdematous tissues, but none other throughout the body; and effecting œdema in

the same way, Hirschfelder, Malmgren and Creavy found that even after the œdema was well developed, mercurochrome and acriflavine given intravenously led to coloration of the œdema fluid.

Hirschfelder applied adrenalin to a rabbit's eye and then some mustard oil. The adrenalin prevented an inflammatory œdema. And yet trypan blue given intravenously became localised in the eye thus treated. The same results followed when cocaine was used instead of adrenalin (p. 176). And Hoff, studying factitious urticaria, observed that if a region of skin was rendered pale with adrenalin and an intravenous injection of congo red was given to the patient, tactile irritation applied to the area of treated skin caused wheals coloured with congo red in spite of the absence of vasodilatation. And in this connection we may recall Lister's observation that stasis and exudation will occur in response to applications of capsicum without any change in the calibre of the bloodvessels.

Hoff and Leuwer noticed that the amount of dye passing into an artificial wheal varied with the varying degrees of pain which were caused. Congo red was injected intravenously and 0·1 c.c. of saline solution was given intradermically. When the pain caused by such an intradermal injection was very slight, only a little congo red passed into the artificial wheal. Using a solution of magnesium chloride which caused more pain, more congo red got into the wheal; while with solutions of potassium chloride, which gave considerable pain, still more of the congo red entered the wheal.

Schmidt found that after exposure to X-rays the connective tissue cells took up increased amounts of trypan blue, while Eckstein and von Möllendorff

noticed an increased colouring of the skin with the same dye after exposure to ultra-violet rays.

Roosen[1] reported the localisation, after intravenous administration, of isamine blue in tuberculous glands and other pathological tissues, and Anitschkov stated that any active hyperæmia would increase the passage of dyes through the vascular endothelium; in fact, the distribution of colloid substances after their intravenous introduction could be quantitatively changed by thermal and other stimuli.

Okuneff[1] applied a hot-water bag to the abdomen of a rabbit and then gave an intravenous injection of trypan blue. As a result not only was the belly wall where it had been in contact with the hot-water bag strongly coloured, but so also were the subjacent coils of the intestine. Okuneff confirms Goldmann's observation that a functioning mamma collects trypan blue. He quotes an article by Kusnetzowsky,[1, 2] at that time unpublished, in which the latter mentions that active local hyperæmia brought about by heat or chemicals will cause an accumulation in the affected tissues of lithium carmine or collargol after intravenous injection of these substances which become stored in the macrophages. With venous congestion caused by pressure, also, there will occur a temporary accumulation of trypan blue and lithium carmine, though storage of these substances in the tissues does not follow passive congestion. He further suggests that these facts may be of therapeutic value.

Hanger[1] made injections of an antigen (filtrate of *B. lepisepticum*) into the skin of a rabbit at hourly intervals. After five hours erythema was present at the site of the earliest injections, but not at the site of

the latest. Indian ink was now injected intravenously, and almost at once the ink became localised at the place where the most recent injection of antigen had been made, while the skin about the earlier injections and elsewhere remained uncoloured. Microscopical examination showed that the Indian ink was in the capillary endothelium.

Kreyberg found that the tarring of mice to produce cancer caused a pronounced local hyperæmia with increased transudation which he rendered apparent by giving intraperitoneal injections of carmine twenty-four to forty-eight hours before the mice were killed, or by killing them with injections of Indian ink. Owing to the increased permeability of the vessels in the tarred area, the carmine or ink becomes concentrated there. This vascular reaction to tarring takes place, Kreyberg states, as long as tarring is continued, from the first application till the appearance of papillomata. After this state and pending the transition to malignancy, there seems to be a gradual obliteration of the vessels in the painted region by multiple capillary thrombi.

Bryce states that, working in his laboratory with guinea-pigs, Dr. Nicol has observed that in the intra-vitam staining of the tissues by trypan blue the uterine mucosa at certain stages becomes densely stained.

Ramsdell used the fixation of trypan blue by inflamed tissue as an indicator of sensitisation. Rabbits, having been sensitised to horse serum, were given an intravenous injection of trypan blue and immediately afterward 0·01 c.c. of horse serum was injected into the skin of the base of the rabbit's ear. The result was an immediate localisation of the dye around the puncture in the ear. A repetition of the experiment two days

after a positive sensitivity test had been carried out failed to effect any localisation of the dye, showing, Ramsdell thought, that desensitisation had occurred.* In normal rabbits she found that histamine, even in a dilution of 1 : 1,000,000, when injected into the skin of the ear, caused localisation of trypan blue at this spot after the dye had been given intravenously.

Spagnol[2] showed that a mere application of chloroform for fifteen seconds to the skin of a rabbit was enough to localise in the area of skin so treated a deposit of trypan blue or colloidal black sulphide of mercury which had been injected into an ear vein. In another paper Spagnol[3] has recorded that, using the method just mentioned, of many dyes injected into the bloodstream, only those which were electronegative became fixed in the irritated area of skin. His results are shown in the following table:

Electropositive Dyes.			*Electronegative Dyes.*		
Dye.	*Dose in g. per kilo.*	*Fixation.*	*Dye.*	*Dose in g. per kilo.*	*Fixation.*
Methylene blue	0·050	–	Alizarin red	0·050	+
Safranin ..	0·020	–	Eosin	0·010	+
Methyl violet ..	0·020	–	Kongorubin ..	0·040	+ +
Thionin ..	0·020	–	Biebricher scarlet	0·020	+
Bismarck brown	0·100	–	Indigo-carmine	0·050	+ +
Brilliant green ..	0·020	–	Indulin	0·020	+
Neutral red ..	0·040	–	Aniline blue ..	0·020	+ + +
Toluidin blue ..	0·050	–	Trypan red ..	0·020	+ +
Gentian violet ..	0·020	–	Trypan blue ..	0·010	+ + +
Nile blue ..	0·005	–	Nigrosin ..	0·020	+ + +
Janus green ..	0·010	–	Alkali blue ..	0·020	+ +
Night blue ..	0·0005	–	Azo blue ..	0·020	+ + +
Victoria blue 4 R	0·001	–	Congo red ..	0·020	+ + +

* It would be of interest to know the results if these experiments were repeated with controls.—H. B.

Menkin and Menkin gave intraventricular injections of trypan blue into frogs, and watched the changes in the concentration of the dye within the mesenteric capillaries in the presence and in the absence of a local aseptic peritonitis. In the frogs with peritonitis the concentration fell about twice as quickly as it did in normal frogs owing to an increased permeability of the capillary endothelium induced by inflammation and a consequent failure of the vessels to retain the dye.

Warren Crowe, investigating arthritis, has made use of the localisation of trypan blue by inflamed tissues to demonstrate disseminated lesions in the bones, joints, and other connective tissues of rabbits following bacterial injections.

Spagnol,[1] using rabbits, brought a loop of intestine out of the abdomen and exposed it to air for a time, varying in different experiments from ten to thirty minutes, and then replaced it. A quarter of an hour later he gave an intravenous injection of a mercury sulphide sol. The animals were killed after three or four hours, and fixation of the mercury was found to have occurred in the exposed viscus.

Roosen has observed and has made use of the fact that isamine blue becomes localised in tissues subjected to diathermy.

The writer, working on vital staining at the Research Institute of the Cancer Hospital, found that Indian ink (Plate I.), trypan blue, trypan red, isamine blue, Congo red, and water blue, when injected into a vein or into the peritoneum of rats and mice became localised not only in the reticulo-endothelial organs, but also to a striking degree in any tissues which happened to be inflamed. Among the lesions producing such localisa-

PLATE III

Localisation of dye around a chronic caecal ulcer

Isamine blue, given intravenously to a rat suffering from a chronic caecal ulcer, has become localised in and about the inflamed caecum.

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tion of dye were a parasitic cyst in the liver of a mouse, a chronic cæcal ulcer in a rat (Plate III.), an ulcer of the palate in a rat with a secondary abscess in the neck (Plate IV.), nodules due to scabies in the ears of rats (Plate IV.), the tissues surrounding the middle ear of a rat affected with otitis media, and operation wounds in rats. Aseptic inflammation set up in the subcutaneous tissues also caused a localisation of dye in the affected region (Plate I.). The irritants used for the latter purpose were agar, gum, kaolin suspended in 5 per cent. gum solution, and Coley's fluid—the latter being used experimentally at the time in the treatment of grafted sarcomata. Application of a diathermy current to a rat's thigh caused a pronounced deposit of trypan blue in those superficial and deep structures of the limb which lay between the electrodes.

Further experiments by the author are as follows.

(1) Mice which had been tarred twice on the back during the preceding week were given intraperitoneal injections of isamine blue and immediately afterwards were again tarred, and bi-weekly tarrings were continued. The dye became localised in the treated areas of skin, though its concentration varied considerably as between the different individuals.

(2) A white rabbit was anæsthetised with ether, and a strip of filter-paper wet with chloroform was held in contact with an epilated patch of skin on the left flank for twenty seconds. A Bier's suction cup was then applied to the epilated area so as to include part of the area which had been treated with chloroform. By means of a suction apparatus connected with a manometer a continuous negative pressure equal to a 4 cm. column of mercury was maintained within the cup;

10 c.c. of a 1 per cent. solution of isamine blue were now injected into an ear vein. The Bier's cup was retained in position for half an hour afterward. During this period no cyanosis was observed in the skin within the cup, the area treated by chloroform showing more brightly pink than the untreated skin within or without the cup. At the end of this half-hour the area of skin to which chloroform had been applied was exactly demarcated with blue except where the cup was applied. Within the circle no deposit of dye was visible (Plate V., B).

It may be added that this experiment was repeated on three occasions using negative pressures within the suction cup equal to 6, 8, and 10 cm. of mercury respectively. In each experiment the result as regards the deposition of dye was as given above—that is to say, where suction was applied no dye became deposited in the skin. Previous observations by Cohnheim and Lewis which bear upon this matter have been mentioned elsewhere (pp. 28, 29).

Venous engorgement brought about by an elastic ligature in the hind limb of a rat failed to fix more than a trace of isamine blue given directly into the bloodstream, although while the congestion was being maintained the treated limb appeared to contain a large excess of the dye.

(3) Repeated experiments in which cutaneous irritants—formalin, mustard, chloroform—were applied through a stencil and an intravenous injection of dye given immediately, have shown that the dye becomes localised only in the skin to which the irritant has been actually applied, and not in the surrounding zone of hyperæmia, which is quite in accord with

Lister's remark that whereas dilatation of arteries occurs over a wider area in the frog's foot than that to which mustard has been applied, stasis is strictly limited to the area of application. Using Sir Thomas Lewis's[2] terminology, it may be said that localisation of a dye in response to irritation of the skin takes place only in the area of local red reaction and not in the surrounding area of the flare (Plate V., A).

(4) Comparative tests have shown that the capacity of an inflamed tissue to localise a dye from the blood-stream is not always proportional to the strength of the reagent which has caused the inflammation. A rabbit was partially shaved and to the bare skin were applied in three separate places 40 per cent. formaldehyde, 20 per cent. formaldehyde, and chloroform, and as soon as these applications had been made some isamine blue was injected into the animal's ear vein. Within five minutes pronounced localisation of the dye had taken place in the skin which had been treated with chloroform, but no such localisation occurred in the area to which 40 per cent. formaldehyde had been applied, and only a slight coloration could be seen in the 20 per cent. formaldehyde region. It should be explained that the chloroform was applied as a standard, because its capacity for localising dyes in the skin of rabbits is regular, pronounced and well known to the writer from experience.

(5) A rat with a large chronic abscess in the neck was anæsthetised with ether and some isamine blue injected into the left femoral vein. The outer necrotic wall of the abscess was then cut off with scissors. The slight oozing of blood which followed soon ceased. The thick pus and debris were now gently wiped away,

exposing the base of the abscess. This was coloured a deep greenish-blue by the injected dye, which was already strongly concentrated here. A strip of white filter paper was now so held that its end rested on the base of the abscess, from which a greenish-blue coloured lymph was escaping. For half an hour this coloured lymph continued to drain away along the strip of filter-paper. At the end of this time the rat was killed by excess of anæsthesia.

It may be of interest to turn from the foregoing direct observations in order to consider some experiments which throw light on the distribution of colloidal dyes following their intravenous injection. A dominant influence in this distribution, it will be seen, is the character of the electrical charge carried by the colloidal particles. Electronegative dyes, with which most of the work on vital staining has been done, become deposited mainly within the reticulo-endothelium of the liver, bone-marrow, spleen and lymph nodes, and also, when the presence of inflammation permits of their exit from the bloodstream, in the macrophages of the inflamed tissue. But they have little or no tendency to enter the parenchymatous cells of the various organs. Electropositive dyes, on the other hand, are not taken up to the same extent by the reticulo-endothelium, but do pass into the parenchymatous cells—for example, the secretory cells of the liver and pancreas—if brought into contact with them.

Two difficulties arise in connection with the intravenous injection of electropositive dyes—namely, (1) their rapid adsorption by the oppositely-charged proteins of the blood and bloodvessels, and (2) their

toxicity. When introduced slowly and in small amounts to the bloodstream, certain of these electropositive dyes undergo a reversal of their electric charge without irreversible precipitation, and in this altered state are conveyed through the circulatory system. Even so, they do not necessarily become distributed exactly like a dye whose original charge was negative—that is to say, almost exclusively to the reticulo-endothelial cells. Vital red, for example, is an electropositive dye, and therefore moves to the kathode when undergoing electrophoresis in distilled water. Added in relatively small quantity to serum or fresh milk and then placed in an electric field, the dye will move to the anode, presumably owing to reversal of its electric charge through adsorption to the electronegative colloids of the medium. Nevertheless, and in spite of this change, vital red does not become distributed in the same manner as a dye which is intrinsically electronegative, such as isamine blue. Ludford produced staining in mice with vital red. Subcutaneous injections of 0·5 c.c. of a 0·5 per cent. solution in distilled water were given subcutaneously on the first, second and third days of the experiment. On the fourth day 1 c.c. of the same solution was given, and the animal was killed six and a half hours later. By cytological methods he found that the acinar cells of the pancreas were stained by the vital red, whereas they are not stained by electronegative dyes.

Owing to the relatively small amounts which can be introduced into the blood of a living animal without causing severe signs of poisoning, the staining of living tissues with electropositive dyes is not as a rule as pronounced or obvious as that which can be brought

about by the less toxic electronegative dyes. Moreover, perhaps because of the reversal of charge already mentioned and due to the adsorption of the dye by oppositely charged colloids, electropositive dyes given *in vivo* are not debarred from ingestion by the cells of the macrophage system. The special capacity of these cells for taking up electronegative dyes is recognised and may be used for cytological identification. By whatever route they are introduced into the living animal, electronegative dyes eventually find their way into the macrophages.

It may be noted as a matter which perhaps carries some pathological significance, that electropositive dyes appear to be toxic not only when injected directly into the bloodstream, where their ill effects might be attributed to precipitation and consequent obstruction of capillaries, but also when introduced beneath the skin, where they cause inflammation.

The mere alteration of its electric charge does not necessarily destroy the toxicity of an electropositive dye. For example, night blue adsorbed to isamine blue remains very poisonous. It is probable that in the circumstances of living tissue the bond between two such mutually adsorbed dyes is not a strong one, and is readily broken. An analogous case is that of radon, which is strongly adsorbed by carbon *in vitro*, but after introduction in this form into the living animal is eliminated with remarkable rapidity.

The apparatus in use at the Cancer Hospital Research Institute for determining the character of the charges on colloidal dyes is one derived with minor modifications from Ettisch and Deutsch. A dry cell battery giving 120 volts is used, the terminals being attached

to two ordinary metal bull-dog paper clips (Fig. 1). Each bull-dog clip is fixed by a screw to a vulcanite slab (A), so that the gripping edges of the two clips are parallel with each other and $1\frac{1}{2}$ centimetres apart. In the space between the two clips is a vulcanite platform (B) on which the preparations can be rested during a test; this platform extends beneath and gives support to the lower, gripping edges of the bull-dog clips (this is not shown in the illustration). An ordinary glass microscope slide is broken into halves, and one of the halves (C) is placed between the teeth of the

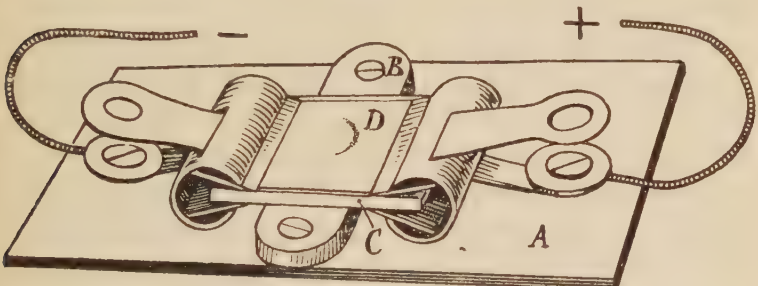


Fig. 1.—Apparatus, modified from Ettisch and Deutsch, for determining the electric charge on colloidal dyes. For description, see text.

bull-dog clips, in which position it is supported by the vulcanite platform. Parchment paper is cut into squares of about the same width as the microscope slide, and these squares are well soaked in a jar of distilled water. To test a dye one of these pieces of parchment paper is taken from the jar of water and partly dried by pressing it firmly between two layers of coarse filter-paper. The slightly damp parchment paper (D) is then laid upon the glass slide together with which it is fixed between the grips of the bull-dog clips. With a pipette a small amount of the fluid to

be tested is now placed on the parchment paper midway between the two clips and the battery is connected. Almost at once the dye will be seen moving out from one-half of the drop to become concentrated in a crescentic area on the side either of the anode or the kathode, according to the character of the charge on the dye particles. When the displacement of the dye has been completed the preparation can be dried and preserved as a permanent record. Droplets of 3 or 4 millimetres in diameter are the most convenient, and a diameter of 1 mm. is not too small for a good reading.

This simple apparatus is of value not only to learn the character of the electrical charge on any particular dye, but to observe the reversal of the charge on an electropositive dye—vital red, crystal violet, toluylene blue, for example—when it is added in relatively small quantity to serum or milk or to another dye which is electronegative. At the same time measurement can be made of the proportional amount of an oppositely charged colloid required to cause precipitation or to reverse the electrophoretic movement of any given dye.

The following example indicates this in a broad way:

	Dye Mixtures.	*Movement of both Dyes in Electric Field.*
(1)	3 c.c. disulphine green 0·33 per cent. .. 1 c.c. night blue 0·05 per cent.	To anode.
(2)	3 c.c. disulphine green 0·33 per cent. .. 2 c.c. night blue 0·05 per cent.	To neither pole (precipitation).
(3)	3 c.c. disulphine green 0·33 per cent. .. 3 c.c. night blue 0· 05 per cent.	To kathode.

Both von Jancso[1] and Spagnol[3] have reported experiments showing that the ordinary distribution of a given dye *in vivo* may be altered by mixing the dye before-

hand with a sufficient quantity of another dye bearing an opposite electrical charge (p. 204).

Keller, who remarks that 99 per cent. of vital staining consists of anode dyeing—owing to the fact that basic dyes are adsorbed *in vivo* to negative colloids and so, like acid dyes, move toward the positive pole in an electric field—points out that many coarse colloidal dyes cannot be easily introduced into cells in spite of their appropriate charge, merely, as he says, because their molecules are too large.

F. P. Fischer has made some interesting observations on vital staining with methylene blue and alizarin S. These dyes belonging to the triphenyl methane series become decolorised after their injection into the blood-stream; but Fischer found that the colour of the dye was restored in certain tissues. Thus he states that the vessels of the iris are electronegative and reduce methylene blue to its leuco-derivative, while the ciliary epithelium, which is electropositive, oxidises methylene white to methylene blue, these powers of oxidation and reduction being lost at death.

Using dyes which do not become decolorised in the body, it is found, he says, that electropositive dyes stain the tissues which oxidise, while the electro-negative dyes stain the reducing tissues. For example, the corneal epithelium, which is an oxidising tissue, is coloured by methylene blue. He also remarks that while an electropositive dye will stain the corneal epithelium, it will not pass through it from without inward, whereas an electronegative dye will pass through but will not stain the cornea. Similarly an intravenous injection of fluorescein, which is electronegative, colours the vessels of the iris and the aqueous humour, while

the ciliary epithelium remains quite unstained. A comparable result was observed in the central nervous system, where it was found that dyes which penetrate the cerebrospinal fluid colour the vessels of the pia mater and choroid plexus, but leave the covering epithelium quite unstained, whereas dyes which coloured the epithelium failed to enter the cerebrospinal fluid.

In these experiments Fischer used albino rabbits and investigated the intraocular staining by means of a slit lamp. He states that it was possible, in spite of the recognised difficulties, to give basic dyes intravenously in such a way as to maintain their concentration in the blood at a sufficiently high level and for a long enough time to effect exudation. He supplies a table showing the results obtained with ten electronegative and ten electropositive dyes. The electronegative (acid) dyes with two exceptions permeated the cornea, entered the aqueous humour and cerebrospinal fluid, and coloured the bloodvessels of the iris, choroid and pia mater, while they left the ciliary epithelium and choroidal epithelium quite unstained. The two exceptions were Chicago blue and diamine black, which failed to permeate the cornea or to enter the aqueous humour and cerebrospinal fluid, owing, Fischer believed, to the relatively large size of their molecules.

The ten electropositive (basic) dyes all failed to permeate the cornea or to colour the vessels of the iris and choroid, while they dyed both the ciliary and choroidal epithelium.

The conditions present would seem to supply a protective mechanism against the entrance of substances which might affect the transparency of the aqueous humour or injure the central nervous system. For

electropositive substances will be adsorbed on to the negatively charged surfaces of the colloids and cells of the blood and bloodvessels, and so will be prevented from reaching the extravascular regions, while negatively charged substances which are able in certain conditions to pass through the walls of the bloodvessels will be arrested by the positively charged surface to which they next become opposed. The process may be compared with the result of dipping the end of a strip of filter-paper into a much diluted watery solution of a dye; the filter-paper, being charged negatively, will hold back an electropositive dye by adsorption, while the water of the solution continues to spread along the strip of paper. An electro-negative dye, on the other hand, will not be adsorbed by the filter-paper, and therefore will be carried along it together with the water.

Keller makes use of the terms " tissue anodes " and " tissue kathodes " to denote the living structures which carry the respective electrical charges in normal circumstances.

Wesselkin used rabbits, injecting various dyes directly into the carotid artery. He found, unlike Fischer, that acid dyes did not enter the aqueous humour or cerebrospinal fluid in health, but a previous puncture of the cornea and withdrawal of some of the aqueous humour allowed trypan blue to enter the chamber, though neither Nile blue nor neutral red entered the aqueous in similar conditions. He found that both Nile blue and neutral red, when injected into the carotid artery, caused staining of the cells of the brain. In the interpretation of this result it has to be remembered that electropositive colloids are toxic, and it may

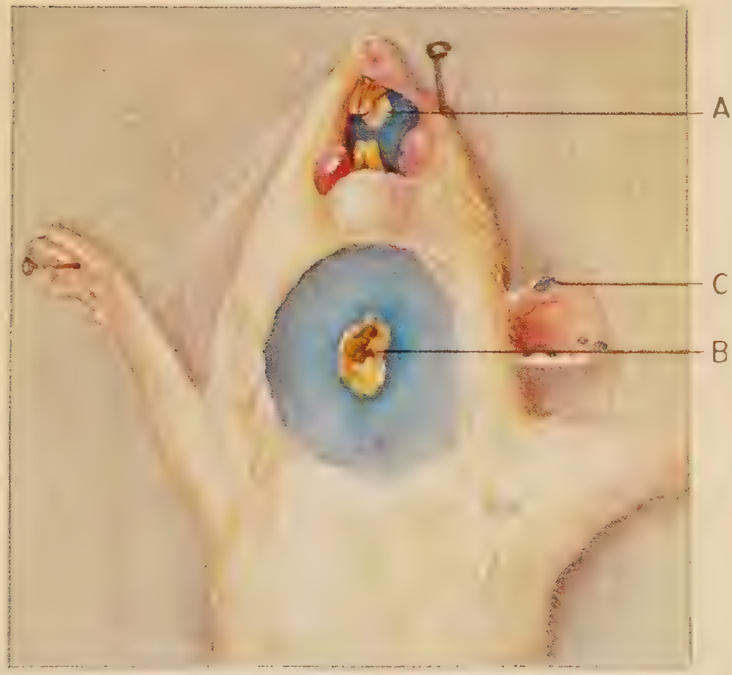
be that, when introduced in relatively high concentration directly into the carotid artery, they penetrate the first defences of the brain—namely, the walls of the cerebral capillaries—by altering their electrical charge and by causing them to inflame.

Starkenstein and Weden, investigating the physiological action of atophan (2-phenyl-chinolin-4-carboxylic acid), found that when given intravenously it caused a modification of the normal distribution of a dye subsequently administered by the same route. The effect of the atophan, they believe, is to alter the electrostatic charge on the tissues in a negative direction, and so to assist the diffusion of an electronegative dye, while impeding that of one which is electropositive.

Electrical influences are not the only ones which regulate the staining of living tissue. The size of the colloidal dye particles is a factor, and so too is their diffusibility. Schulemann remarks that the best dyes for vital staining are those which diffuse in gelatine with medium velocity. Rous, Gilding and Smith also have studied the effects of differences of diffusibility on vital staining.

It should be added that the distribution of coloured matter in the living body is not dependent upon any special chemical action. All colloid particles obey the same general rules as those which hold good for dyes. Schulemann found that palladium, gold, platinum and silver, when injected in colloidal form, were taken up by the same cells which take up trypan blue. Spagnol[1,2] made similar observations with regard to mercuric sulphide. Menkin[2] noticed that a ferric chloride sol both in normal conditions and in the

PLATE IV



Localisation of dye in an ulcer, an abcess and in nodules of scabies.

A rat with an ulcer of the palate and a secondary abcess in the neck was given an intravenous injection of isamine blue. The dye has become concentrated in the ulcer (A), abcess (B) and in some nodules (C) caused by scabies in the margin of the ear.

[*To face page* 80

presence of inflammation obeyed the same laws of distribution which govern the colloidal dyes, and numerous workers, including the writer, have made similar observations with Indian ink and many other coloured substances.

One curious and perhaps significant fact is reported by von Jancso[2], namely, that colloidal metals are not taken up by the reticulo-endothelial system if, just previous to their injection, one of the anti-coagulants—heparin, novirudin or germanin—is administered. Hirudin he found without influence in this respect.

CHAPTER VI

THE LOCALISATION OF NATURAL PIGMENTS

Natural pigmentation of the human skin seems to be governed by the same laws which regulate the deposition in the skin of dyes and other substances from the blood as brought about in the laboratory. But in saying this two special factors have to be carried in the mind. First, the natural process as it occurs in man is usually spread over a longer period of time, while the tissue stimulation is of a gentler sort than in most of the experimental work that has been recorded. Secondly, certain districts of the human skin appear to be more easily put in a condition to receive pigment than others. These districts of easy pigmentation will be referred to as susceptible areas.

Perhaps objection may be made against the inclusion of cutaneous pigmentation in this discussion on the alleged ground that the pigments concerned are not brought along by the blood, but are formed locally by the irritated cells of the skin. This explanation of pigment formation may or may not be correct. On the other hand, the localisation of pigment in the skin fits in so aptly with the localisation of dyes, viruses, and micro-organisms, that to omit skin pigments from the argument in the absence of a complete knowledge of their origin would seem to me unfortunate. Furthermore, if cutaneous pigmentation in response to irritation is to be accounted for by the inflammatory fixation

of locally-formed colouring matter, its consideration is still germane to this essay.

Stimuli that lead to Local Deposits of Pigment.—Special factors which lead to the deposition of natural pigment in certain places are irritations of various kinds. Ultra-violet light is the first that comes to mind, and the sequences of a sunburn illustrate what happens—namely, a local inflammation in the parts of the skin exposed to the light, followed by pigmentation of those parts and those parts only. Heat is another localiser of pigment. The best example of its action is perhaps the condition known as *erythema ab igne*, which occurs on the fronts of the legs of some people who are much exposed to the heat of an open fire. Here again there is first a chronic redness of the skin, followed in the course of time by pigmentation; although, compared with sunburn, the process is a slow one. Large doses of X-ray and radium also can bring about a dermatitis followed by pigmentation. Pressure, friction, and other mechanical irritants have the same effect. Individuals belonging to that large section of mankind that is doomed to wear collars almost constantly show a band of darker-coloured skin corresponding to the annoyance caused by the garment. At other places where pressure and friction are common, pigmentation occurs—over the prominent spines of cervical vertebræ, over the *tuber ischii* of people who sit much on hard seats, and on those parts of an amputation stump which transmit the patient's weight to the artificial limb.

Lacapère and Laurent have reported, with photographic illustrations, a curious and unexpected example. They noticed that most of the male inhabitants of Fez had on their foreheads a strongly-marked pigmentary

tache due to striking their foreheads many times a day on the "natti" of the mosque. As is mentioned elsewhere, gummata are apt to appear in these pigmented patches on the forehead (p. 93).

Scratching and other frequent or long-continued mechanical stimulation will lead to local deposits of pigment, and so will the application of chemical irritants, as, for example, the application of a mustard leaf, which will often result in a striking patch of pigment of the exact shape and size of the leaf that was used. Chronic infections are potent localisers. Long-standing ulcers of the leg demonstrate the fact well, and in these the greatest concentration of pigment is often to be seen, not in the scar itself, but in the tissues immediately adjacent to the scar.

The significance of pigmentation is better understood if it is appreciated that the amount of colouring matter laid down is not proportional to the severity of the causal "inflammation." A sudden acute lesion leading quickly to stasis and followed by resolution is not so effective towards the deposition of coloured matters in the inflamed tissues as will be a gentler irritation which is just sufficient to bring about increased permeation of the capillaries without causing stasis or any violent reaction. The chronic irritation associated with scabies provides a good illustrative instance, the pigmentation of the spots produced by the acarus being especially conspicuous. Rats suffer from a parasite which burrows in the skin of their ears and other parts where the hair is scanty or absent. On injecting isamine blue or trypan blue into a rat so infested, the dye will become deposited in the affected

nodules in sufficient quantity to colour them a distinct blue (Plate IV.).

Another example of the localisation of pigment by irritation is afforded by urticaria pigmentosa. The following details are abstracted from the notes of a case shown by Parkes Weber at a meeting in London of the Royal Society of Medicine. The patient was a woman of twenty-six years. The skin of her whole body and limbs was studded with brown macules. These macules, which had begun to appear five years previously and had ceased to increase in number for some time, were not elevated, and could not be distinguished from the rest of the skin by touch. But on being rubbed they became bright red and swollen, and then commenced to itch. Apart from the spots there was no factitious urticaria. In this case, as in others of a similar nature, it seems probable that pigment becomes deposited in the spots, together with the plasma which transudes from the bloodvessels whenever these sensitive areas of the skin give an urticarial response to mechanical stimulation.

The general distribution of pigmentary deposits in conditions of ill-health have been carefully studied in connection with arsenical poisoning and Addison's disease. And for practical purposes there is little difference to be found between the two disorders in this respect. Méneau noticed that when patients with psoriasis had been treated with arsenic they not infrequently showed local pigmentation in the areas affected with psoriasis and also in areas where psoriasis had been recently cured. Kelynack and Kirkby found that arsenical pigmentation had a tendency to localisation:

(1) In those regions in which pigmentation occurs

naturally—that is to say, the axillæ, the nipples and their areolæ, which even in men might become nearly black, the umbilicus, the perineum, the genitals, and the anus.

(2) In areas of skin exposed to the weather.

(3) In skin which is subjected to friction and pressure—for example, over the ischial tuberosities, prominent vertebral spines, and over the shoulders. In a woman patient whom they examined pigmentation was most marked around the waist, and also in a band around each leg corresponding to the pressure of her garters.

They give a good description of reticular pigmentation. In this, "the groundwork of the skin," they say, "is deeply pigmented, but small areas, varying in size from $\frac{1}{16}$ to $\frac{1}{8}$ inch, are correspondingly lighter in tint than the adjacent portion of skin, and some of them seem to be quite free from pigment. The appearance presented conveys the idea that the pigment, having first been deposited evenly, has been washed out by small drops of water." Brooke and Roberts have also remarked upon the tendency of arsenical pigmentation to appear in any irritated part of the skin, and among other situations they mention the neighbourhood of old scars. Since that time Borsellini and numerous other observers have recorded the coincidence of arsenical pigmentation with pressure and irritation of the skin from a variety of causes.

Susceptible Areas of Skin.—Examples of these are (1) the cheeks, and (2) the darker areas of a marbled skin. In most skins there is a certain amount of mottling, which becomes obvious if the conditions are suitable; but in some individuals the mottling or marbling is more pronounced than in others. It has

an anatomical basis as was first shown by Renaut, who observed that when a blue mass is injected into the vessels of the skin the mass first appears at isolated points in the skin. These blue points, as the injection continues, gradually enlarge until at last they coalesce. Each little vascular territory of the skin, he says, is commanded by a small artery which is distributed in the shape of a cone with its base directed toward the free surface of the skin. Such a distribution, he remarks, gives a clue to several phenomena. According to his idea, the paler areas of a mottled skin represent the regions of the best arterial supply. This view, in its broadest significance, is accepted by Lewis, although he has not been able to confirm the results obtained by Renaut on injecting the vessels of the skin. Lewis[1] has shown that the areas of mottling are constant in any given individual. The paleness, he finds, does not depend upon any difference in the number of vessels which are present. "I have often counted," he says, "the capillaries in adjacent pale and dark zones to find that they are equal in number, and that the arrangement and number of the venules is the same in both. The difference in the depth of colour is due to difference in the size of the vessels, and this is explained by differences in tone." In the pale areas, tone is considerable. A moderate increase of venous pressure therefore heightens the contrast between pale and dark areas. Although in repeated observations Lewis was unable to detect any but negligible and inconstant differences in the temperatures of the redder and paler areas, in an extreme case of *erythema ab igne* the average number of readings placed the temperature of the pale areas 0·08° C. above that of the darker reticular mesh-

work. Lewis has found that the vessels of the darker meshwork in a mottled skin are, like those of the cheeks, relatively " atonic," and react more slowly and less completely to histamine, adrenalin, and pituitrin when these drugs are pricked into the skin with a needle.

Adamson,[2] in a paper on livedo reticularis or mottled skin, makes the following remarks upon the condition:

" (1) The dusky marbling or network known as livedo annularis is a result of the tuft-like arrangement of the bloodvessels of the skin as demonstrated by Renaut. The network corresponds to the regions of ' reduced circulation ' between the tufts.

" (2) It occurs as a normal condition in many children and young adults, and is exaggerated as a result of exposure to cold.

" (3) In departure from health due to tubercle, syphilis, rheumatism, alcoholism, hypothyroidism, etc., the normal livedo annularis may become exaggerated and persistent. . . .

" (4) In such persons with persistent and exaggerated livedo the position and pattern of eruptions due to syphilis or tubercle may be determined by the livedo, so that the eruption takes a network aspect and may be called ' inflammatory livedo.'

" (5) A similar inflammatory livedo, with subsequent pigmentation, may be produced by prolonged exposure of the skin to heat—' livedo a calore.'

" (6) The distribution and arrangement of certain eruptions, such as those of secondary syphilis, of measles, of parakeratosis variegata, and perhaps of others, is explained by the anatomical arrangement of the bloodvessels of the skin which gives rise to livedo annularis."

The fact that pigment is especially apt to become localised in these darker reticulations has been remarked upon by a number of different observers. Unna

noticed such selective localisation in the pigmentation which precedes sailors' carcinoma and xeroderma pigmentosum; Kelynack and Kirkby, Borsellini, Lehner and Kenedy,[2] Montgomery and Culver, have all reported this reticular pigmentation in connection with arsenical poisoning, while its striking manifestation as a consequence of heat in *erythema ab igne* is generally recognised.

The opportunity here offered may be used to remark that not all pigments show a ready tendency to settle in the skin where this is irritated. In ochronosis, although the cartilages of the nose and ear may be so deeply stained as to show like dark shadows beneath the integument, the skin itself may display little or none of the pigment. But an exception like this does not affect the general question, especially when we consider that the pigment of ochronosis differs from the substances with which this essay deals inasmuch as it does not become taken up by the cells of the reticulo-endothelial system, or, if taken up by them, does not retain its colour.

Unexplained selective affinities exist between particular tissues on the one hand and various substances—including alkaloids, toxins, pigments, viruses and bacteria—on the other. With this group of facts the present essay is not directly concerned, its sole aim being to demonstrate a particular mechanism by which colloids and discrete substances can pass through the unbroken walls of bloodvessels and so come into close contact with the extravascular tissues.

To sum up, it may be said that, provided pigment is available, it will become deposited (1) in any part of the skin which is subjected to continued but not too

violent stimulation, and (2) in certain areas of skin which are naturally susceptible to such deposits, including the axillæ and scrotum. And as in different patients the areas of skin stimulated will vary according to whether the patient is up and about or confined to his bed, exposed to ultra-violet light or not so exposed, and a number of other circumstances, so will the deposits of pigment in his skin vary in their localisation. But the circumstances of the patient and the distribution of the pigment will always be in logical accord.

One other point has to be mentioned. The areola of the nipple during pregnancy becomes the seat of much pigmentation. Goldmann, as already mentioned, has found that isamine blue becomes deposited copiously in the areolæ of the nipples of a pregnant mouse. We must therefore add certain physiological activities to the list of localising agencies in the fixation of pigment, bearing in mind in this connection the vital dyeing of the uterine mucosa, the placenta, and fœtal membranes, to which reference has been made on former pages.

An interesting speculation is to consider what part may be played in dermatitis by an increased sensitivity to light caused by the localisation of hæmatoporphyrin in the affected skin. That this pigment sensitises the skin to sunlight there is experimental proof; that it will become deposited in irritated tissue there can be little doubt. There are, therefore, sufficient reasons for suspecting that the deposit of hæmatoporphyrin in exposed parts of the skin under the influence of minor degrees of irritation may be the starting of a vicious circle, and may be responsible for some of the more pronounced cases of intolerance to sunlight.

CHAPTER VII

THE LOCALISATION OF SYPHILIS

THE *Treponema pallidum* is a satisfactory organism to study so far as concerns its fixation in tissues which are inflamed, because, unlike many bacteria, the Treponema grows better in such a nidus than elsewhere. Old granulating wounds, as Billroth long ago demonstrated, and numerous experimenters—Afanassieff, Noetzel,[1] and many others—have since confirmed, offer a greater resistance to most bacterial infections than that offered by fresh wounds. Yet granulations and recent scars constitute a favourable soil for the inoculation of *Treponema pallidum*—a fact which Chesney and Kemp, and Halley, Chesney, and Dresel have proved by trials on animals. Not only will this organism infect a granulating wound when applied directly to its surface, but it will become localised there and initiate a lesion even though introduced into another part of the body. Perhaps the most convincing experiments of this kind were some carried out by Chesney, Turner, and Halley. Skin was excised from two places in the backs of five rabbits, the wounds being then left to granulate. Twenty-two days later, these wounds having healed, each rabbit was inoculated in the right testicle with 0·1 c.c. of a testicular emulsion containing *Treponema pallidum* in virulent form. Between the twenty-seventh and the forty-eighth day after these inoculations indurated, syphilitic lesions appeared in both dorsal scars of all the animals so treated. With the exception

of a papule in the skin of the right foreleg of one of the rabbits no other cutaneous syphilides were observed.

In another series of four rabbits a piece of skin was excised from the back as before and the wounds left to heal by granulation. Twenty-two days later, when all but one of these wounds had healed completely, a second piece of skin was removed from another part of the back. Eight days after the latter operation all four animals were given intravenously 0·1 c.c. of a testicular emulsion of active treponemes. At periods varying from nineteen to sixty-seven days subsequent to these injections, every one of the dorsal scars became affected with indurated syphilitic lesions, whereas no other cutaneous lesions occurred.

The same observers record two human cases bearing upon this point, and illustrate their article with photographs. In the first, burns were sustained on both forearms and the left arm. Three months later the patient became infected with syphilis, and at the end of another three months there were definite cutaneous syphilitic lesions localised about the scars of the burns. In the second case a negro, who had keloid scars on the face, trunk, and right arm resulting from razor slashes sustained between one and two years previously, acquired syphilis, and six months later had generalised skin lesions, these syphilides being especially numerous and pronounced around the keloid scars.

Clinically it has long been a recognised fact that syphilitic rashes are apt to crop up in territories which are already occupied by sores due to some other cause. And as long ago as 1843, Cazenave laid down the rule that the locality of a secondary syphilitic lesion is

commonly determined by some concomitant irritation or morbid condition.

Tarnowsky, in 1877, was so deeply impressed by observing that local irritation of the skin in a luetic subject would often bring about the appearance of a syphilide at the irritated spot, that he was led to introduce what has been known since as the "Tarnowsky test" for syphilis. This was carried out by applying mustard paste to the patient's skin. A positive result was an indurated lesion arising in the treated area after the total disappearance of the reactive symptoms evoked by the application of the mustard. His test most often yielded positive results in the early stage of syphilis, and especially if it happened to be applied just before the secondary rash came out, in which case a positive reaction was almost always obtained. Negative results could be ignored, but positive results were reliable.

The special tendency of the earlier general manifestations of syphilis to appear in areas of irritation may be taken in these days as an accepted clinical fact. Possibly there may be some scepticism as to whether the later syphilitic developments often are dependent upon some injury for their appearance. There is a medico-legal side to this question which lends it additional importance, and which has been dealt with particularly by Stolper and Klauder. That gummata may be consecutive to injury seems certain. Lacapère and Laurent, in a paper already quoted, stated that among the Moroccans gummata of the middle of the forehead were so common that some special cause must have been responsible. And they came to attribute these curiously placed gummata to the Mussulman

custom of touching the ground with the forehead during prayer. Five times daily this exercise is performed, with a total duration of half an hour a day.

Viets thinks it beyond question that trauma plays an etiological part in neurosyphilis, and mentions the case of a man who received a superficial shell-wound over the mid-thoracic region of his spine. A few weeks later he had signs of a complete transverse myelitis at the segmental level of the cord which corresponded to the superficial wound. Both blood and cerebro-spinal fluid yielded a positive Wassermann test, and the symptoms cleared up rapidly under antisyphilitic treatment.

With regard to the influence of trauma in promoting syphilis of the brain, it is not easy to obtain convincing evidence. Some of those who have been in the best position to form an opinion on the subject believe that injury can play a sinister part: v. Krafft-Ebing states definitely that localisation of syphilis in the brain may follow as the result of overwork, excesses, and physical disturbance; he refers also to similar effects of alcohol, heatstroke, and injury. Mott also believed that cerebral trauma could lead to the more rapid progress of general paralysis, especially if the accident were associated with commotion or concussion. Osnato supports the view that general paralysis may be a consequence of cerebral injuries, and he gives illustrative cases, among which the following is a good example. A man fell the height of three stories, sustaining a linear fracture of the vault of the skull, fracture of six ribs, and numerous contusions and abrasions. In the following month, the patient, who denied ever having had syphilis, began to mumble and to suffer from hallucinations and

delusions. Later he had atrophy of the left optic nerve, delusions of grandeur, euphoria, restless activity, visual hallucinations, and great intellectual and emotional deterioration. The fracture of the skull was evident in an X-ray photograph. His blood and cerebrospinal fluid both gave a strongly positive Wassermann reaction. When we consider the experiments of Macklin and Macklin, already quoted, in which they found that a dye given intraperitoneally came to be localised in the rat's brain as the result of concussion, we can more readily accept the clinical evidence as maintaining the general theme when applied to cerebral syphilis in man.

Syphilis and Tattooing.

Here may be a convenient place for referring to the numerous cases which can be found in medical literature of syphilitic rashes, in the generalised stage of the disease, appearing on old tattoo marks. Zechmeister seems to have been the first to publish examples. He described two cases, one from his own clinic and another the details of which had been supplied to him by Róna in a personal communication. In Zechmeister's case a syphilitic papular rash appeared in a tattooed area a fortnight after the operation. In Róna's case a man who had been tattooed on the arms three years previously was admitted to the clinic with generalised syphilis. The rash, in his case, was widely distributed, but was greatly intensified in the designs on his arms. Wechselmann reported two examples. A patient with the figure of a policeman tattooed on his forearm had secondary syphilis the rash of which became specially concentrated about the tattoo marks

so as to outline the whole figure with a broad band, and to raise it up above the level of the skin. In another case a man had tried to get a tattooed design on his forearm obliterated, so that it was hardly visible. He became infected with syphilis, and during the secondary stage of this disease the dye particles became everywhere surrounded by small papules, so that the original tattoo figure became recognisable as a Red Indian furnished with a headdress. Lipschütz had a patient in whom a recurrent syphilitic rash was present in a tattoo mark and nowhere else. Florange reported two examples and had seen many similar ones. His first case was a man of twenty-nine with syphilis contracted three months before. He had been tattooed some years previously with a blue and red pattern. Papules were numerous in the blue tattoo marks and absent from the red. His other case was a man of twenty who had taken syphilis eight weeks before. Some years earlier he had been tattooed, also with blue and red, but in this case all the papules were on the red areas and none were on the blue. A portion of the red tattooed skin was excised and found to contain cinnabar. In Dohi's case, with which is published a fine coloured illustration, the tattooing had been done sixteen years previously. Syphilitic papules were present in the blue areas and were more numerous in the heavily shaded blue areas, but no papules occurred in the skin which had been tattooed with cinnabar. A piece of this skin was excised and the presence of cinnabar was verified. Holland, who described four cases and had seen many others of a similar kind, said that when a syphilitic rash broke out in an individual who had been tattooed, eruptions in the areas tattooed red were the

exception, while eruptions in the blue areas were the rule. Buschke published two pictures illustrating the localisation of syphilides in tattoo marks. Bernheim and Gluck had a particularly interesting case. A man of eighteen years had numerous tattooings done on his chest and arms. A year later he was infected with syphilis. Eleven months after becoming infected he introduced into the old tattoo marks some red ornamentation with cinnabar which he had obtained from a chemist. A fortnight or so afterwards a recurrent syphilitic eruption broke out which involved only the recent tattoo marks. These were covered with rash, whereas the rest of the body, including the old tattoo marks, red and blue, was quite free. Aoki had a patient who, two years before contracting syphilis, had been tattooed with the design of a snake, the materials used being Chinese ink and cinnabar. Numerous secondary syphilitic papules appeared regularly along the lines of the blue tattoo marks, being especially profuse on the deep blue-black portions, while the markings made with cinnabar remained free from rash. Keidal and Zimmermann also record a case in which syphilides were localised in tattoo marks and vaccination scars. They remark that, although they have examined a very large number of syphilitic people with tattoo marks, this is the only case in which they have seen selective localisation of the rashes on areas which have been tattooed. They, however, accept it as well recognised that the localisation of secondary and tertiary syphilitic lesions is often determined by irritation and trauma.

In the previous chapter reference was made to the liability of the darker areas of a mottled skin to become

the seats of pigmentary deposits. The *Treponema pallidum* likewise becomes localised in these areas. Neumann, Ehrmann, and Adamson[2] have noted that syphilis is especially apt to appear in these darker areas of reticulated skin, an observation which has received additional support from Lehner and Kenedy,[1] and Williams and Goodman.

Erasmus Wilson, in his "Portraits of Diseases of the Skin," and under the title of Melanopathia Syphilitica, has an illustration of the leg of a young married woman of twenty-three years with pronounced reticular pigmentation. From the appearances the picture would pass for a good example of the deposition of pigment following *erythema ab igne*. But the domestic conditions were those of poverty, and Wilson could not obtain any history of exposure to the heat of a fire, though he inquired particularly into this possibility.

Some observers have been so much impressed by the relationship between syphilides and the darker parts of a skin affected by *livedo reticularis* that they have gone so far as to attribute the livedo itself to syphilis.

CHAPTER VIII

THE LOCALISATION OF BACTERIA

THE evidence available to prove that bacteria are sometimes collected from the bloodstream by inflamed and otherwise altered tissues is conclusive. Clinical instances are numerous and striking. The phrase *locus minoris resistentiæ* is hoary with years, and the initiation of local infective processes by injury unaccompanied by direct infection is a widely recognised fact. Laboratory experiments support clinical observation in this matter.

BACILLUS TUBERCULOSIS.

Schuller injected tuberculous material through a tracheotomy wound into the lungs of dogs and rabbits and at the same time injured, by twisting or bruising, one of the knee-joints. A generalised tuberculosis followed, together with a local tuberculous inflammation of the damaged joint. Krause injected pure cultures of tubercle bacilli subcutaneously into guinea-pigs and intravenously into rabbits, and, either immediately before or after, broke a bone or wrenched a joint. No tuberculosis followed at the sites of fracture, but in one-half of the rabbits and one-third of the guinea-pigs the injured joints became tuberculous. Salvia introduced virulent cultures of the tubercle bacillus intravenously into rabbits and at the same time he applied trauma to various parts of the body, and he

found on several occasions that injured bones became affected with tuberculosis.

Many clinicians have called attention to the frequency with which tuberculosis arises in regions already affected through some other agency. As long ago as 1878, Jonathan Hutchinson[1] noticed that lupus was very often associated with chilblains and other abnormal conditions of the skin caused by exposure to cold and heat. "The connection of *lupus erythematosus*," he says, "with the same tendencies which evoke chilblains, is illustrated by the fact that the parts affected are, for the most part, those which suffer most from exposure to cold—for instance, the nose, ears, and hands. *Lupus erythematosus* is very rarely seen in those parts of the surface which are constantly protected by clothes. It is also made worse by exposure to wind and cold, and very often there is a history of actual chilblains having occurred on the ears. The conditions are, however, although allied, not identical, and exposure to heat is as injurious to these lupus cases as is cold. Sunburn of the nose is a common exciting cause."

Since Hutchinson's original observations were made several clinicians have directed attention to the fact that cutaneous tuberculides are particularly liable to appear in skin which has been the site of some previous non-tuberculous trouble. Colcott Fox, at a meeting of the Dermatological Society of London, showed a case in which the tuberculous skin lesion known as *erythema induratum* was associated with chilblains; MacLeod, at another meeting of the same Society, brought forward a patient who was suffering from (i.) chilblains of the hands and feet, (ii.) *erythema*

induratum on the calves of both legs, and (iii.) *Lupus erythematosus* of the face. Adamson,[2] Lehner and Kenedy,[1] and Williams and Goodman, have remarked upon the tendency of tuberculides to appear on the darker areas of a reticulated skin—a characteristic localisation to which reference has already been made in connection with syphilis and natural pigmentation; it will call for further attention when discussing the rashes of measles.

Haxthausen gives several examples of the same sort. He agrees with other writers (*vide* Hallam) that sufferers from tuberculosis are especially liable to suffer also from perniosis, in which term he includes *cutis marmorata*. At the same time he states that perniosis—which is described as a chronic change in the skin induced by cold—encourages the development of cutaneous tuberculides, and particularly of that form known as *erythema induratum*, in which condition " very frequently the nodules are to be observed confined just to the centre of the skin area showing the most intensive perniotic changes." Other tuberculides, including lupus, he says, are apt to appear in skin which has been damaged by exposure to cold, aided, it may be, by sunlight. At a meeting held at the Royal Society of Medicine, MacCormac showed a patient of twenty-six with *lupus erythematosus*. In addition to the lesion upon her cheeks and nose she had eruptions on the backs of her fingers and on a V-shaped area where the skin of her chest was exposed. Well-marked tuberculous glands were present in the neck, and MacCormac regarded the abnormal cutaneous condition as tuberculous. The case appears to exemplify Haxthausen's observations mentioned above.

At the same meeting at which the previous case was shown, Brain exhibited a case of lupus occurring in a vaccination scar. The patient was a girl of twelve who had been vaccinated in the arm eighteen months previously, when two pustules resulted. One of these healed normally, but the other remained sore, and the upper and lower crescents of this scar showed the apple-jelly infiltration characteristic of *lupus vulgaris*. The girl had slightly enlarged cervical glands, but no other sign of tuberculosis. The father, who was living at home, had been suffering from phthisis for seven years. Both of these cases appear to the writer to be explainable on the basis of a localisation by diapiresis of tubercle bacilli from the bloodstream into irritated tissues.

Unlike many, and perhaps most, pathogenic micro-organisms, the tubercle bacillus appears to find a favourable nidus in hyperæmic and inflamed tissues where its destructive action is amplified. Haxthausen, discussing the incidence of lupus on skin which has been injured by cold and exposure to sunlight, makes the following statement:

"The facial area of skin showing these combined light and cold effects constitutes without any doubt a *locus minoris resistentiæ* in many cases of *lupus vulgaris*, having a hæmatogenous origin. Thus the first lesions may be observed very frequently to arise as one single or some few nodules in the centre of the red area in the middle of the cheek, and this dependent state may be still more often recognised by observing the *extension* of the lupus over the skin of the face. Suppose a case of lupus commencing on the nose and spreading over the cheeks, it may often be noticed that the narrow pale area separating the redness of the cheeks from that

of the nose is completely or almost entirely spared, and it is only when the disease reaches the prominent area of the cheek with its changed vessels that the nodules become larger and more numerous. It is in this way that the "*butterfly*" *pattern* is produced, so characteristic of many lupus cases where the disease has spread from the nose on to the face. In the case of men with whiskers and beards it may be observed sometimes that that part of the face covered by the beard and so relatively spared the action of cold and light is similarly spared the extension of a lupus affection."

That the likelihood of curing a tuberculous bone or joint is diminished once a secondary infection has become superimposed upon the previous lesion is hardly to be denied; and the evil prognosis of phthisis when engrafted upon silicosis is widely recognised.

With a general tuberculous infection an inflamed tissue forms a true *locus minoris resistentiæ*, allowing first a localisation of the bacillus and secondly a nidus which, owing to hyperæmia aided perhaps by an increased hydrogen ion concentration, is favourable to the continued growth of the micro-organism. The evidence that a decrease of alkalinity is an accompaniment of inflammation will be adduced later (p. 197).

Thus silicosis not only causes fixation of the tubercle bacillus in the lung, but by maintaining an inflammatory hyperæmia it provides the bacillus with a favourable medium. Out of this fact have arisen large and difficult economic problems which have not yet been overcome. The therapeutic bearings of these matters will be discussed more fully in a subsequent chapter (p. 259).

Gye and Kettle injected (1) silica, (2) tubercle

bacilli, and (3) silica and tubercle bacilli under the skin of mice, and they found that the lesions produced by the combination of silica and tubercle bacilli were more vigorous than those brought about by tubercle bacilli alone.

Later Kettle reported further experiments. He found that if a local subcutaneous inflammation were produced by introducing calcium chloride or turpentine, and the animals were subsequently inoculated intravenously with tubercle bacilli, these became localised in the inflamed tissues. He further obtained a similar fixation of the tubercle bacillus in subcutaneous lesions effected by silica. But whereas the inflammation set up by calcium chloride or turpentine was more severe than that caused by silica, the fixation and proliferation of the tubercle bacillus was much greater in the lesions caused by the silica. This is in accordance with the clinical and experimental information collected in this volume; that is to say, the gentler irritants may be the more effective agents in causing localisation. In other words, localisation does not bear a direct proportion to the degree of irritation.

Bacillus Lepræ.

The bacillus of leprosy appears to follow the same rules for localisation in the skin as does the bacillus of tubercle. Hopkins, Denney, and Johansen made a careful investigation of the distribution of lesions in cutaneous leprosy, and in a survey of three hundred and two lepers they found that there were certain regions in which the macules, infiltrated patches, and nodules of skin leprosy rarely if ever occurred. "These areas are among those which are less exposed to irritation from

such sources as sunlight, heat, cold, pressure, friction, and other causes." The regions found to be immune in all patients were the posterior inferior auricular area, the concha, the orbital side of the nose near the inner canthus of the eye, the lateral palpebral area external to the outer canthus, the axilla, the inframammary fold in women, the interdigital surfaces, and the perineum. The scalp, nasolabial fold, and the sulcus of the upper eyelid were found involved in only two, four, and two cases respectively. Their paper contains good illustrative photographs.

Bacillus Welchii.

Kettle's experiments on the localisation of the tubercle bacillus by injections of silica and other tissue irritants call to mind some rather similar ones carried out by Bullock and Cramer, who inoculated washed suspensions of *B. welchii* and also of *Vibrion septique* under the skin of normal mice and also of mice which had received at a distant spot a subcutaneous injection of a soluble calcium salt—calcium chloride, calcium nitrate, calcium citrate were used. The results were that the bacteria themselves alone, washed free from toxin, did not cause lesions. In the mice which had been treated with calcium lesions appeared; not, however, at the spots where the bacteria had been injected, but at the remote localities which had received the calcium. In other words, the bacteria had been taken up by, and fixed in, the tissues which had been inflamed by the calcium.

These results are, perhaps, the counterparts of those cases in which an emphysematous abscess has come about at the site of a subcutaneous infusion. Wanke

has collected twenty-five examples of this phenomenon which became familiar to surgeons during the Great War, and was apt to be attributed to a defective technique, an explanation which Wanke accepts in his paper as applicable to most of the cases.

In some of these instances it may have been that the pathogenic bacteria were not localised immediately from the bloodstream. They may have been lying latent in the tissues until aroused into activity by some suitable stimulus. At any rate, this appears to be the explanation of some results obtained by Andrews, Rewbridge, and Hrdina. They were investigating the occurrence in dogs of lesions due to infection with *B. welchii*. Their experiments may be summarised as follows:

(1) Dog bile introduced into the chest or pelvis led to acute infection with *B. welchii*.

(2) Intramuscular injections of sterile bile salts or liver extracts in most cases caused gas gangrene within eighteen hours.

(3) Dog's muscle ground up and injected into the peritoneum caused peritonitis and usually death within twenty-four hours.

(4) Autoclaved ground muscle given intraperitoneally was followed by no ill effects.

(5) Tight ligation of a limb with wire so as to cut off the arterial supply led to simple gangrene without gas formation, odour, or positive culture in most cases.

(6) Similar ligation, accompanied by the injection of sterile liver extract, was followed by emphysematous gangrene.

B. welchii would appear from these experiments to have been present beforehand in the muscles of the

dogs used, although the failure to produce emphysematous gangrene in experiment (5) does not seem to accord well with such a conclusion.

That living organisms after lying dormant for long periods of time in the tissues may be awakened into sudden pathogenic activity is recognised; and the fact must not escape mention in a consideration of the mechanisms that govern local infection. The recrudescence of inflammation in tuberculous joints as a sequel to injudicious movements, or the flaring up of sepsis in old wounds after surgical interference, are recognised examples.

Bacillus Typhosus.

Keen, in his *Surgical Complications of Typhoid Fever*, published in 1898, remarked upon the powerful influence of mechanical causes as the proximate factors in causing these complications, and he quotes a case in which the patient, having recovered from typhoid fever, received a blow on the forearm which was followed by an abscess of the ulna. The most interesting of these metastatic abscesses are those which have followed hypodermic injections, because with these the circumstances are known with exactitude. Moreover, unless the affair is understood, the mishap may be attributed erroneously by the patient or his friends to a want of aseptic precautions in using the hypodermic syringe. Catrin described two examples in which abscesses occurred at the sites of hypodermic injections given to patients who had typhoid fever. Schneider had a similar case in which the abscess had followed the use of chlorhydrate of quinine, the pus of this

abscess containing Eberth's bacillus in pure culture. Achard and Weil quoted two instances in which the *B. typhosus* was recovered from abscesses following hypodermic injections of methylene blue given to patients in the course of typhoid fever. Burdach had a similar case following a hypodermic injection of camphor. Widal and Le Sourd had under their care a patient who, during convalescence from typhoid fever, suffered from an abscess and three subcutaneous collections of oily fluid exactly at the places where hypodermic injections had been given. Typhoid bacilli were isolated from the pus of the abscess and also from the oily collections. Lesieur found *B. typhosus* in the pus of a fixation abscess induced in a typhoid patient. Rathery and Bonnard reported a similar experience, and referred to another one in which a typhoid abscess followed a hypodermic injection of quinine sulphate. In a discussion which followed the report of these two cases Netter stated that a colleague was actually appearing before the tribunals at that very time because abscesses had developed in two of his patients at the spots where hypodermic injections had been made by a certificated nurse. Willimczik mentioned another instance of a typhoid abscess forming at the site of hypodermic injection—camphor and caffeine had been used; and Madelung gives a collection of similar cases which he has found in the literature.

Widal and Ravaut have described two especially interesting examples of the localisation of typhoid bacilli by incidental lesions:

(1) A young woman, who had suffered from tuberculosis of the submaxillary lymph nodes for many years, became ill with typhoid fever. During con-

valescence the nodes suppurated and the pus was found to contain the *B. typhosus* in a pure state.

(2) A woman of thirty-four had typhoid fever. After recovery from this she complained of abdominal trouble. A laparotomy revealed an ovarian cyst containing 1,500 grammes of fluid from which a pure culture of the typhoid bacillus was obtained.

Rathery and Bonnard give an account of a girl, aged sixteen, who was operated upon for appendicitis. Three days later she was found to have typhoid fever. The operation wound healed by first intention, but twenty-four days after its infliction an abscess was found under the scar. This was opened and the pus evacuated was found to contain the *B. typhosus*.

Bottema mentions the case of a patient suffering from a carbuncle in whom a large abscess developed at the site of an intramuscular injection of quinine. He has observed a similar sequence when quinine injections have been given in cases of furunculosis, abortion, and typhoid fever, but never when the same treatment has been used for malaria.

Benians has made some experiments on this subject. He injected various substances (gum tragacanth, mucin, starch, agar, McConkey's bile-salt agar, and muscle extract) into the subcutaneous tissues of rabbits and then injected bacteria into the ear vein. He found that *B. coli* (non-motile form), *B. typhosus*, and *B. paratyphosus* readily became localised in the injection mass and were isolated therefrom. *Staphylococcus aureus* usually could not be so recovered, and *M. catarrhalis* and streptococci were not recovered at all. Egg albumin and mutton fat failed entirely to localise bacteria from the bloodstream.

PNEUMOCOCCI.

The pneumococcus, like the typhoid bacillus, readily becomes taken up by inflamed tissues, in which it may cause suppuration. Zuber[1, 2] described a case of pneumonia in which abscesses containing pneumococci occurred at the sites of hypodermic injections of caffeine benzoate, and he was able to collect a great many similar cases from the literature. Méry and Bonsaude had a patient with pneumococcal pneumonia in whom abscesses appeared at the sites of caffeine injections, the pneumococcus being found in the pus. Two similar cases have been recorded by Guinon and Bureau and one by Achard and Weil, in which pneumococcal abscesses developed at the sites of hypodermic injections of caffeine given to patients with pneumonia.

Rolly infected mice with pneumococci and found that their lives were prolonged when inflammation and abscesses were produced by injections of a silver salt or of turpentine.

STREPTOCOCCI.

Rolly also has recorded, in the same paper, a case of severe puerperal sepsis in which the patient received twenty-four subcutaneous injections of a silver preparation in the lower limbs. Abscesses developed round the punctures. The patient recovered. In another case streptococci were grown from the patient's blood and also from the pus of abscesses which had been caused by hypodermic injections in the same manner as those mentioned above.

Frank describes the cases of two children, aged five and six years respectively, who suffered from scarlet

fever. Each of them was given, on the second day of the illness, 25 c.c. of scarlet fever immune serum, which was injected into the buttock. Four days afterwards abscesses had developed at the sites of the injections, and from these abscesses hæmolytic streptococci were isolated in pure culture. Strict attention to asepsis had been observed while making the injections.

Frank gives also the clinical notes of an infant of two years who suffered from pneumonia, and on the fifth day of this disease showed the symptoms of scarlet fever. The principal features of the case may be tabulated briefly as follows:

April	20.	Pneumonia.
,,	25.	Scarlet fever.
,,	30.	120 c.c. of pus containing pneumococci in pure culture withdrawn from right pleural cavity.
May	20.	Child apparently well.
,,	31.	Empyema of right pleural cavity; 20 c.c. of pus withdrawn containing hæmolytic streptococci in pure culture.

Faber injected into the knees of rabbits a *Streptococcus viridans* which was only slightly pathogenic for rabbits. When the resultant arthritis had subsided, that is to say within two to four weeks, he gave an intravenous injection of the same organism, and in this way was able constantly to produce an arthritis in the previously treated knee. A possible interpretation of this result seems to be that a residual inflammation in the primarily infected joint had led to fixation in that region of streptococci introduced in the bloodstream in the second stage of the experiment. Such is not Faber's own interpretation.

Sager and Nickel performed the following experiment on seven rabbits. After epilation and sterilisation of

the abdomen, subcutaneous abscesses were initiated by injecting $1\frac{1}{2}$ or 2 c.c. of a 10 per cent. aqueous solution of silver nitrate. At the same time blood was withdrawn from the heart and cultured, and found to be sterile in every case. Between six and ten hours after the subcutaneous injection of the silver nitrate, green-producing streptococci were injected intravenously. Twenty-four hours later the blood was found to be sterile. After four days fluid was found to have collected at the sites of injection of silver nitrate. This fluid was aspirated and examined, and in every case the streptococcus which had been introduced into the blood was found to be present in the inflammatory exudate, whereas in six control animals treated otherwise in the same manner, but omitting the injections of streptococci, the exudate caused by the silver nitrate remained sterile.

In another five rabbits silver nitrate solution was injected subcutaneously as before, but no bacteria were introduced at first. Fixation abscesses formed and were found to be sterile. Streptococci were now given intravenously, and in two of the five rabbits these streptococci were subsequently recovered from the abscesses.

Kinsella and Sherburne, experimenting with dogs, were able to show that if an aortic valve was injured by an instrument passed through the left carotid artery and an intravenous injection of a culture of green streptococci was then given, the bacteria became implanted on the injured valve.

The Fixation Abscess.

Hippocrates observed the favourable effect which local abscesses seemed to have upon the prognosis in certain diseases, and recognised the value in treatment of hot fomentations and counter irritation.

Jenner[2] was a firm believer in the efficacy of artificial inflammation in diverting disease from vital parts.

"May we not," he asks, "by making new diseases, check the progress of disease in a vital organ or in a part where it may be unmanageable, by substituting another which is under our control? . . . Whoever has observed the deranged state of health where vesiculated eruptions have been called into action by an effort of Nature, must have seen how often they arrest the progress of the original disorder, and may we not from thence infer what appears to me to be a pretty general law of Nature, that she often gets rid of diseased action affecting vital organs, by exciting eruptions in other parts not vital. . . . I am aware that this doctrine is not entirely new; but though the phenomena have been so often described, have we taken the hint in our treatment of diseases either chronic or acute?"

Jenner gives references to earlier literature on the subject, and describes his own method of treatment, which consisted of the gentle and repeated rubbing of a stimulating ointment into the skin of the inner sides of the arms or other convenient area, until an eruption resembling herpes had been produced in the treated parts. The prescription for his ointment is as set out below:

Antim. tartrat. (subtil. pulv.), ʒii.
Ung. cetacei, ʒix.
Sacchari alb., ʒi.
Hydr. sulph. rub., gr. v.
M. Ft. ung.

An increased proportion of tartrate, he says, will cause earlier inflammatory results.

In spite of the fact that belief in the efficacy of counter irritation as a remedial agent has endured so long in tradition and has been so firmly upheld by leading minds in the profession, the principle involved appears never to have played a dominant part in therapy—possibly because its mechanism was never fully understood. A great deal of new interest was attracted to the subject when Fochier[1] published his paper on the treatment of puerperal infection by subcutaneous phlegmon. The fundamental observation which led him to adopt this method was the sudden amelioration which sometimes came about in a general infection coincidentally with the formation of a localised abscess. Fochier still has many followers in his own country, and particularly in his own district of Lyons, but relatively few elsewhere—the ancient proverb about the prophets being thus inverted. Good results have been claimed for the method on purely clinical grounds in a variety of ailments—puerperal fever (Fochier, Gonnet, Madrids), various kinds of septicæmia and severe sepsis (Snapper, Jacob, Villaret *et al.*, Boidin, Lesné *et al.*, Espenel), pneumonia (Conor, de Lostalet, Destefano *et al.*, Todd, Queyrat), encephalitis (Netter,[1] Martin), Malta fever (Roziès), malaria (Carles[2]), a virus septicæmia following a rat-bite (Brodin and de la Rivière), and various other illnesses, including severe Jacksonian epilepsy (Ricaldoni), poisoning by toadstools (Pic and Martin), and by mercuric chloride (Aquino). Some of this therapy at first may seem bizarre, but not so if considered in the light of such clinical and experimental knowledge as can be derived

from a study of deliberate fixation abscesses, and also of those accidental fixation abscesses which have followed hypodermic injections and local lesions due to other causes.

Fochier[2] infected rabbits with anthrax, and into some of these he injected subcutaneously in a different spot 0·25 c.c. of turpentine. All of the controls died in the usual time—*i.e.*, about sixty-six hours. But some of the rabbits treated with turpentine survived, while others which died yet outlived the control animals.

Giuca, using guinea-pigs infected with trypanosomiasis, produced fixation abscesses by injecting turpentine into the outer part of the thighs. This treatment was followed by a great reduction of the trypanosomes in the blood and sometimes by their complete disappearance—though they returned later. The diminution in number of the circulating trypanosomes was manifest usually within forty-eight hours or less after the injection of the turpentine.

On the other hand, Le Guyon gave lethal doses of anthrax bacilli and diphtheria toxin to different batches of guinea-pigs, and in half of each batch he instituted turpentine fixation abscesses immediately afterward with 0·4 c.c. of essence of turpentine. All the animals died in the same time. He also inoculated mice with virulent streptococci and virulent pneumococci, and to half of each batch he gave 0·2 c.c. of turpentine subcutaneously. As in the previous experiment, the turpentine abscesses did not modify the progress of the infections. Rabbits and guinea-pigs inoculated with bovine tuberculosis showed no alteration in the fatal course of the disease when treated by means of a

turpentine fixation abscess. In view of these experimental results, Le Guyon doubts whether the fixation abscess has any value in the treatment of human disease. His results are striking, but it might be well if some of the experiments were repeated with smaller dosages so as to allow a certain percentage of survivals among the control animals; in this way any effect towards increasing the survival rate by means of a fixation abscess could be more distinctly observed.

An experimental difficulty in connection with the isolation of micro-organisms from a turpentine fixation abscess is the fact that the turpentine, which is an antiseptic, remains in the abscess.

Findlay,[2] in a study of the hæmatogenous infection of wounds in rabbits, used the following method. Bacteria to be tested were given intravenously and immediately afterward flank injections were made of a histamine solution (pH=7·2) on the right side and a phosphate buffer solution of the same pH on the left, these injections being repeated at three-hourly intervals. In the tissues treated with histamine, but not in the control areas treated with phosphate solution, he was able to verify the localisation of *Staphylococcus aureus* (three strains), *Streptococcus hæmolyticus* (one out of two strains), and a pneumococcus.

That the placental site after labour may be a *locus minoris resistentiæ* to infection seems probable, not only because the maternal placenta is shown, by the experimental use of dyes in animals, to have permeable capillaries, but also by the readiness with which sepsis may come about in the uterus of women after labour in circumstances which seem to preclude a direct infection. The following is an example recorded by

Frank. A woman at the full term of pregnancy became ill with scarlet fever. Parturition occurred on the following day. Two days later the patient suffered from acute septic fever. Hæmolytic streptococci were obtained in pure culture from her blood and from the cervix uteri. No vaginal examinations had been made in this case for several months before labour or during labour.

An interesting example of the localisation of bacteria by mechanical injury is quoted by Walker from Chauveau, who states that in some parts of Europe, instead of castrating cattle, it is customary to perform torsion of the testicle so as to rupture the spermatic cord. Fatty degeneration of the testicle ensues, but inflammation never follows. Chauveau found that if active septic material were previously introduced into the circulation, the torsion always led to local suppuration.

As will be shown later (p. 216), inflamed tissues not only segregate bacteria from the blood, but they retain them, and prevent their direct spread to the neighbouring parts.

CHAPTER IX

THE LOCALISATION OF VIRUSES

JUST as no doubt can be held concerning the localisation of bacteria from the bloodstream by inflamed tissues, so none can be entertained as to the localisation of viruses.

MEASLES.—Hebra noticed that if a patient during the prodromal stage of measles had been lying for some time on one side the rash was apt to become apparent first in the arm upon which his weight had been resting, and he further observed that if ointments, plasters, or lotions had been applied to the chest, the rash would often first show itself in that region. The rash also, he remarked, was very prone to appear in any part which was compressed by tight bandages or articles of clothing.

Schick was impressed by the effects produced by tuberculin given shortly before an attack of measles. His case was as follows: The patient, who had suffered from pleurisy, was being given intradermal injections of tuberculin. Before the 3rd March, 1910, ten such injections had been made. On the 3rd, 7th, and 10th March the doses were repeated. On the last-named day the patient had pyrexia. At this time the skin, where the earlier pricks had been made, was marked merely by pigmentation, while the redness and swelling of the last reaction spot were already showing an abatement. On March 15th Koplik spots were seen and a rash appeared behind the ears and on the

face. At this time all the tuberculin sites, except those of the 7th and 10th March, became red and swollen; and in a few hours there appeared at the periphery of these areas obvious changes which could be regarded only as an eruption of measles, and within the areas the rash became confluent, the diameter of each spot infiltrated in this way being greater than the diameter of the original tuberculin reaction.

On the second day of the rash the eruption decreased in intensity, though additional macules appeared at the periphery of the tuberculin reaction areas. The character of the generally distributed rash, though not pronounced, was typical. On this day a copious rash appeared on the latest tuberculin sites—those of the 7th and 10th. On March 15th—that is to say, on the first day of the measles rash—a tuberculin injection was given, but gave no inflammatory reaction. Another dose given on April 5th gave a "strongly positive" response.

In commenting on this case Schick calls to mind that infants with intertrigo get the first rash of measles in the reddened areas, and that with serum rashes, as with *erythema post-scarlatinosum*, the situation of the rash is determined by the preceding inflammation. It was well known, he adds, to older doctors that bandaging or the application of iodine could lead to a copious outbreak of spots if these applications were made during the prodromal stage of smallpox.

v. Pirquet in a classical paper discussed the distribution of the rash in measles with great care, and came to the opinion that the spots appear in all chronically hyperæmic areas earlier than in the normal skin of the environment, no matter what the cause of the

hyperæmia. As examples, he mentions intertrigo, and skin which has been compressed by tight garters. Urticaria and *erythema multiforme* affecting the limbs prior to the outbreak of measles may reverse the order of the eruption of the rash, which now comes out on the limbs before it can be distinguished on the face. Anæmic scars remain poorer in rash than the surrounding skin, though the tissue immediately adjacent to the scar may show a particularly rich crop of spots. As an example, he shows the photograph of a young child whose vaccination scars, almost bare of rash, are each encircled by macules.

Experimentally, v. Pirquet found that he could influence the distribution of a measles rash by a variety of cutaneous irritations provided that they were made in the prodromal period of the illness and at least one day before the general eruption came out. Thus a mustard plaster applied to a patient's leg during this stage led to the outbreak of a confluent eruption over the entire area so stimulated. Mere bandaging of a limb was followed by an early appearance and an increased profusion of spots. Venous congestion caused a delay in the appearance of the maculae, followed by a rich crop.

Adamson[1] noticed that the rash of measles became especially profuse in the darker areas of a reticulated skin, and his observation has been confirmed by an exact piece of work carried out by Lewis and Harmer. They mapped out carefully the mottling of an area of skin in a child's thigh, and the distribution of a measles rash was noted within this district. Of sixty-three macules they found that only three appeared wholly within the pale areas.

Smallpox and Vaccinia.—Hebra stated that when a person previously affected with any other acute or chronic skin disease—eczema, psoriasis, syphilis, for example—was attacked by smallpox, the exanthem developed with peculiar intensity on the parts that were already unhealthy.

Tièche also says that in variola the rash appears copiously where any irritation of the skin is present, and that in these areas vesicles are often confluent so as to give the appearance of a burn. Ordinarily the rash is least abundant in the regions of the body most protected from irritation—the loins and abdomen, for example.

Bénard, Camus, Carnot, and Teissier made a series of experiments relative to the local effects of ultra-violet light upon patients with smallpox. As early in the patient's illness as possible a single irradiation lasting thirty minutes was given to the subject's fore-arm by means of a mercury vapour lamp at a distance of 40 cm. from the treated surface. The characteristic erythema following irradiation was not constantly observed in these cases. Apart from irradiation, the cases were treated in the manner customary at the hospital in which the patients lay. No recrudescence of the smallpox eruption occurred in the irradiated areas; nor did any harmful result follow. If the suppurative stage had been already reached the applications of ultra-violet light exerted no effect on the ulterior evolution of the eruption, but applied in the papular stage the irradiation arrested the development of the lesions and so lessened the subsequent scarring. In every case this favourable action appeared to be dependent upon the appearance of erythema following

the irradiation. In this experiment the absence of localisation of pustules by the irritation caused by the ultra-violet light may have been due to the relatively late stage reached by the disease at the time of the applications. (See v. Pirquet, p. 120.) The fact is mentioned as it may not seem to be in perfect agreement with other observations on the localisation of viruses. Moreover, somewhat similar discrepancies appear in connection with the localisation of vaccinia, under the influence of irradiation, as will be seen. The beneficial effect of the hyperæmia in limiting the secondary pustular infection in smallpox can be understood in the light of general principles (p. 255).

Jenner[1] remarked upon the fact that if children suffering from skin lesions were vaccinated in the arm, typical vesicles not infrequently appeared on the affected parts of the skin. "I have seen many instances," he says, "where pre-existing pimples have been converted into vaccine pocks which have kept pace with those on the arms in their progressive changes."

A curious occurrence—which seems to be quite in line with the observations of Jenner just quoted—has been recorded by Denney and Hopkins. They vaccinated 118 lepers and 105 non-leprous attendants with the same lymph, and found that among the lepers not only was the local vaccinial response severe and often accompanied by necrosis and ulceration, but general fever was caused together with an acute, though temporary, exacerbation of the leprous lesions throughout the body. No abnormal effect was produced by the vaccine upon the attendants who were free from leprosy. Observations on the effects of vaccinating lepers have been reported also by Hasseltine, and

are in agreement with those of Denney and Hopkins.

In explanation of the above occurrences it seems reasonable to suggest that the vaccinia virus may have become localised by the chronically inflamed leprous lesions, a widely distributed, though modified, vaccinial eruption being thus brought about.

The writer has seen in an infant who was circumcised and vaccinated on the same day, in circumstances which almost certainly precluded direct contamination, a lesion appear in the scar of the circumcision wound which might certainly have been interpreted as a modified vaccinial pock, for, although no suppuration occurred, the small localised swelling ran a course approximately parallel with the vaccinial pock on the arm. No sutures or ligatures had been used in performing the circumcision, the wound of which healed by first intention.

Work in the laboratory has amply substantiated the fact that vaccinia virus becomes localised in irritated tissue. Calmette and Guerin, when experimenting on rabbits with the virus of vaccinia, found that plucking out fur from a rabbit, or shaving part of its skin, caused the virus after intravenous injection to become localised in the epilated area and in that area alone, provided that the removal of the fur was done within the first twenty-four hours after the injection. If an interval of forty-eight hours elapsed between the injection of the virus and the epilation, no localisation of the virus occurred in the denuded area.

v. Prowazek and Yamamoto, experimenting with rabbits, epilated a portion of skin with calcium hydrosulphide, and then sandpapered the epilated area and

scarified it. Thirty minutes before this 3 c.c. of vaccine lymph had been injected intravenously. As a result there appeared a confluent lesion in the treated area of skin, and with extracts of the skin so affected they were able to produce typical vaccinial lesions on the corneas of normal rabbits.

Gins and Weber, Ledingham, and Watanabe have confirmed this experimental localisation of vaccinia as an occasional and inconstant sequence. Other investigators have been more strongly impressed. Camus[1, 2] has carried out an experiment which recalls that of Rogowicz, to which reference has already been made. He resected 2½ inches of the cervical sympathetic nerve of a rabbit, thus causing a pronounced vaso-dilatation in the ear. Two and a half hours after dividing the nerve he gave an intravenous injection of vaccine virus. Four days later a vaccinial eruption appeared in the denervated area, and nowhere else. The buccal eruption was so severe that the animal could not eat, and lost 420 grammes in four days. Camus has also noted the tendency of vaccinial virus, when given intravenously to rabbits, to become localised in recent scars, on nævi, on shaved skin, about small burns, in areas of skin to which chloroform had been applied, and also in regions affected with chronic eczema. To get consistent results Camus found that large doses of virus must be given. Incidentally he observed that scratching the skin added nothing to the influence of the razor. Vesicles were often absent from the scratch marks, and in any case they did not show any special tendency to appear along these lines.

Gins is quoted as having produced vaccinial keratitis in rabbits by giving the vaccine intravenously and

subsequently scratching the cornea with a blunt instrument.

Levaditi and Nicolau verified the fact that after intravenous injections of vaccinial virus into rabbits the subsequent cutaneous eruption appeared particularly in areas where the skin had been irritated by pulling out hairs or by other means. Vaccinial lesions of the eye were produced in a similar way by scarification of the cornea or exposing it to X-rays prior to an intravenous administration of the virus.

Duran-Reynals effected localisation of vaccinial virus by means of chicken embryo cultures and chicken sarcoma cultures. Rabbits were injected in different places with the embryo and the sarcoma cultures; the vaccine virus was given intravenously. Vaccinial eruptions appeared in twenty-five out of the twenty-nine places where either of the cultures had been injected. Peptone and kieselguhr used in the same way failed as localisers. The relative times of injecting the localiser and the virus are not mentioned.

Hoffman and Duran-Reynals observed that if an extract of testicle was given intracutaneously and vaccine virus intravenously, the subsequent virus infection became sharply localised to that part of the skin into which testicular extract had been injected.

Andrewes, using a very potent strain of Levaditi's neurovaccine, found that, when given intravenously to rabbits, the virus regularly gave rise to pocks on plucked, epilated or even unshaved areas of skin; but they were always most numerous on plucked areas, where an almost confluent eruption was sometimes seen.

Douglas, Smith, and Price noticed that after a skin

inoculation secondary pocks showed a tendency to localise in skin areas injured by inoculation of control material which was known to contain no virus.

Findlay[2] injected vaccinia virus into the ear vein of each of four rabbits and then gave histamine into the right flank and phosphate solution into the left, as in the bacterial experiments to which reference has been made already (p. 116). As a result he obtained vaccinial lesions in the areas of the histamine injections, and the ground-up skin thus affected produced typical vaccinial keratitis when inoculated on to the corneas of fresh rabbits. At the site of the phosphate injections a single papule appeared in one rabbit, but none in the others.

From the foregoing evidence it appears to be proved that the virus of vaccinia becomes localised in skin which is inflamed. There are certain observations, however, which do not quite accord with such an unreserved generalisation. Carnot, Camus, and Bénard (*cf.* p. 121) inoculated rabbits with doses of vaccine which ordinarily would produce confluent lesions and also with doses calculated to produce discrete lesions. Parts of the skin so inoculated were then irradiated with ultra-violet light. An eruption failed to come about in the irradiated regions, while it appeared normally in the non-irradiated areas. A similar inhibition of the vaccinial eruption was seen when, instead of applying the ultra-violet light to the skin immediately after vaccination, the application was made on the second or third day. The irradiation used was a forty-five-minute exposure to the light of a 3,000 candle-power quartz mercury vapour lamp at a distance of 30 cm. from the skin. This dosage

produces erythema. It is sufficient also to destroy the activity of the vaccine *in vitro*, though such a direct destructive effect does not account for the resistance shown in the experiment, for a similar resistance ensued if the skin was irradiated before inoculation.

Le Fèvre de Arric obtained comparable results following the use of X-rays. A rabbit was epilated and five days later the epilated area was exposed to X-rays, the dose being 5H distributed over an area of about 200 cm. without filtration. Fourteen days later, after light scarification, an inoculation of vaccine was made in the irradiated animal and in a non-irradiated control rabbit. In the irradiated rabbit sixty pustules of small dimensions appeared, whereas in the control there were a hundred well-developed pustules. Even more pronounced were the inhibitory results obtained with X-ray dosages of 7H and 10H, the other conditions of the experiment being as before. Thus a rabbit was treated in the manner just described, except that the dose of X-rays was 7H. On the sixth day after this exposure pigmentation of the skin appeared. Fourteen days after the irradiation vaccine was inoculated into the pigmented skin and also into the neighbouring unpigmented, non-irradiated skin. As a result there appeared in the irradiated area three small pustules only, while in the normal skin the pustules were numerous and nearly confluent.

Rivers, Stevens, and Gates vaccinated rabbits after exposing shaved areas of skin to ultra-violet light. In one group of experiments the rabbits were inoculated within an hour after single exposures; in these animals there was a pronounced inhibition of the vaccinial lesions—that is to say, there were fewer and less severe

pustules than resulted from a similar vaccination of control rabbits. No virucidal property was found in an emulsion made from the irradiated skin. In other rabbits an interval varying from twenty-four to seventy-two hours was allowed to elapse between a single irradiation and vaccination, the skin at this stage being red and œdematous; and now, in contrast with the results of the previous experiment, the vaccinial lesions were accentuated as compared with those of vaccinated, non-irradiated controls. Daily irradiations with ultra-violet light, repeated for a fortnight and followed at intervals varying from one to forty-eight hours after the last exposure by vaccination, showed that the areas of skin so treated possessed an enhanced resistance against the formation of vaccinial lesions. The daily irradiations in the experiment at first caused reddening and desquamation of the skin, after which no macroscopic changes took place, so that, when the vaccination was performed, the irradiated skin was normal in appearance.

These results do not in any way prove that the virus of vaccinia is not localised under the influence of inflammation; the non-appearance of the characteristic pustules may be due to other causes than an absence of the virus. It will be observed that in these experiments with irradiations the vaccinial eruption was diminished in every instance in which the skin was not hyperæmic at the time of vaccination, whereas in the one instance in which the inoculation was performed in hyperæmic skin the lesions were accentuated.

Varicella.—Swoboda refers to the localisation of the rash of chicken-pox by bandages and tight clothing.

Rivers and Tillett[1] give details of some cases which

illustrate the influence of irritation on localising the rash. These instances are briefly summarised below.

Case 1.—An adhesive plaster had been applied to the ankle. The patient developed varicella. The adhesive plaster was removed, and in the area over which it had been applied there were more vesicles than on all the rest of the body.

Cases 2 *and* 3.—Napkin rashes were present, and the varicella vesicles were mostly localised in the erythematous area.

Case 4.—In this instance the patient had worn a soft collar with a tightly pulled tie. He became affected with chicken-pox, and a band of vesicles appeared encircling the neck.

Case 5.—A sufferer from acne acquired chicken-pox, the vesicles of which were confined to his face and involved the actual spots of acne.

In a later paper Tillett and Rivers report another case. A patient with secondary syphilides took chicken-pox, the spots of which became localised in the skin areas affected by the syphilis, being most numerous on the face, scalp, and extremities instead of on the trunk.

Tièche agrees with the general statement that the eruption of chicken-pox is apt to be localised by irritation, but not, he thinks, to the same extent as the smallpox rash.

Netter,[2] supporting the hypothesis that varicella and herpes are connected, has referred to cases in which herpes zoster has followed tuberculosis of the spine, erysipelas, and syphilis, and in three cases the eruption followed an injection of novarsenobenzol or bismuth. "The serum of these patients was found to give, without exception, a fixation in the presence of the antigen of varicella." In Netter's cases we are re-

minded of the Tarnowski test for syphilis, in which a latent disease is rendered manifest by local cutaneous irritation.

Scarlet Fever.—Perhaps it is not strictly in order to discuss scarlet fever in a section devoted to virus infections. But we are dealing with fever rashes, and the liberty will be taken of giving the disease a very brief consideration here, because the distribution of the rash in scarlet fever follows the same general rules of localisation as have been found to hold good for the rashes of fevers due to viruses.

Hebra long ago pointed out that sometimes, instead of its usual distribution, a scarlet fever rash would appear first on regions of the body which had been kept warm, " such as the loins, back, abdomen, elbows, and knees "; " Like the other exanthemata, too, scarlatina affects first and with most intensity parts which have previously been exposed to pressure or friction."

Heim and John, under the title of " The Reinflammation of a Cutaneous Reaction that had already passed away, during an Attack of Scarlet Fever," have published a case which has interesting bearings on the general topic now under discussion. On 13th March a child of four years was given three doses of different tuberculins into the flexor surface of the left forearm. There was a strong reaction which began to subside after six days, finally being represented by three pigmented flecks. On 23rd March regular tuberculin treatment was commenced, and between this date and 10th April four injections were made on the extensor surface of the forearm. These injections excited no general nor local reaction. On 13th April the child fell ill with scarlet fever, having a temperature of 104°,

a sore throat, and a typical scarlet fever rash on the face, neck, and chest; the abdomen and extremities being free from rash at this time. But to their great astonishment Heim and John found on the flexor surface of the left forearm a reinflammation of the three spots where the inoculations had been made a month previously. All three spots suddenly came up "as though up till now they had been under the skin." On the 15th April the rash had spread over the whole body, "even the revived cutaneous papules are surfeited with it." On this date the later tuberculin injections given between 23rd March and 10th April showed pronounced local reactions with reddening and infiltration.

ADDITIONAL VIRUSES.—Flexner and Amoss tested other ways of infecting animals with poliomyelitis than by applications of the virus to the nasal mucous membrane or by introducing it directly into nervous tissue, and they discovered that the disease could be brought about by intravenous injection, but only when relatively enormous doses of the virus were given. If, however, an intraspinal injection of foreign serum (horse, monkey, man), or even of isotonic salt solution or Ringer's solution, had been made twelve or eighteen hours beforehand, infection was readily established by giving the virus intravenously.

The most exact and detailed experiments that have been carried out upon this general subject are perhaps those of Maitland, who proved that the localisation of the lesions of foot-and-mouth disease in guinea-pigs is due to pressure and slight traumatism. By bandaging the foot of an animal suffering from this disease in cotton-wool, for example, he was able to prevent any lesions from appearing on that foot, though its un-

protected fellows all suffered from the typical lesions. The foregoing facts, as elicited by Maitland, appear to be beyond question; nevertheless it is remarkable that the virus of this disease should become localised in response to such relatively slight and normal stimuli as the pressure on the feet when standing and the small injuries received by the tissues of the mouth during the apprehension and mastication of food. Such minor traumata, if the functional forces mentioned can be so described, apparently are insufficient to bring about the localisation of most bacterial infections. It is possible that the discrepancy may be due to the smaller size of the virus as compared with bacteria; for the ease with which diapiresis is effected appears to depend to some extent upon the effective size of the particles concerned. A corollary would be that the smaller the particle the less will be the degree of permeability required for its localisation. Carrying the matter a step further, it may be supposed that viruses will be localised by lesser stimuli than are required to bring about the localisation of bacteria. Such an argument is not altogether vague guesswork. There is collateral evidence in favour of the idea, including the mild applications which have been noticed by various observers to cause the localisation of the rash in measles, smallpox, varicella, and other virus diseases. However, this hypothesis must remain in a nebulous condition until the facts have been condensed by experimental inquiry yet to be carried out. The relatively slight causes which lead to the localisation of pigment will be recalled in this connection. The matter will come up for discussion later (p. 202).

Teague and Goodpasture put forward evidence

suggesting that herpes simplex and herpes zoster are manifestations of a disease due to a single causal agent; and some of their observations have a bearing on the present matter. They inoculated the previously tarred skin of guinea-pigs and rabbits with material from human herpetic vesicles, and produced a zonal herpes with lesions of the corresponding root ganglia. The irritation of the skin caused by the tar was necessary, they say, for the appearance of a zoster-like eruption, although a local non-zonal "take" might be induced on an untreated skin.

Lewis[2] recalls that herpes zoster is due to a lesion of the ganglion on the posterior root of a sensory nerve, and he suggests that antidromic impulses set up by such a lesion cause a release of vasodilating substance in the skin which is thus prepared for localisation of the virus. It appears to be almost certain, as already pointed out, that hyperæmia alone causes but little if any increase of exudation; consequently, without additional factors, it is hardly likely to bring about a vesicle. Nevertheless, a hyperæmia brought about as Lewis suggests might lead to the exudation necessary for the formation of a vesicle provided that some slight lesion or defect were already present in the area of skin supplied by the affected nerve root. Any condition responsible for the formation of a vesicle would be favourable at the same time to the localisation of a virus from the bloodstream, a vicious circle being thus established. Notwithstanding the observations of Camus (p. 124), it is doubtful if hyperæmia alone would cause the localisation of a virus.

In connection with the foregoing paragraphs it is

of interest to regard a discussion which took place in Paris in 1873. Verneuil had described three types of traumatic herpes and had attributed the lesions to nerve injuries. His typical cases were as follows: (1) A nerve is wounded in its continuity and herpes occurs within the distal area of distribution of the nerve. (2) A nerve is divided and herpes appears in the distal area of its distribution. (3) Herpes follows a wound of a nerve, but not in the area which the wounded nerve supplies. At a subsequent meeting, however, Parrot put forward the view that a latent general infection of herpetic fever had been present in such cases and had become localised as a result of damage to the tissues. He pointed out that in one of Verneuil's cases the patient—a child—had vesicles not only at the site of a scar, but on the lips also. In measles and smallpox it was well known, he said, that when the eruption appears it tends to come out in areas which have been irritated. Head and Campbell described several cases in which an eruption indistinguishable from that of herpes zoster accompanied lesions of the posterior root ganglia caused by sarcoma, fracture of the spine, and tuberculous caries. Netter's observations, to which reference has been made in the discussion of varicella (p. 129), may be considered again here as possibly supporting Parrot's explanation of this kind of case.

As remarked already, though hyperæmia by itself has little effect in causing exudation, when associated with an increased permeability of the capillaries, it leads to a pronounced increase of exudation. This being so, we may suspect that hyperæmia not only assists and accelerates the localisation of viruses from the blood-

stream, but that it facilitates also the production of vesicles.

Tumour-producing Viruses.—While experimenting with epithelioma contagiosum of fowls, Burnet learned that plucking out some of the feathers led to the appearance of tumours in the denuded area after intravenous injection of the virus. If one pulled out a single feather a tumour appeared at that point. If the bird were completely plucked it would become covered with tumours. He noticed that the longer the interval between the injection of the virus and the denudation the less was the intensity of the resulting local lesions. Dealing with the same disease, Goodpasture remarks that the one great factor determining the localisation of the virus with the resultant formation of a lesion is epithelial injury, whether the virus is applied externally or whether it circulates in the bloodstream. Findlay[1, 2] was able to cause local lesions in pigeons by injecting histamine into the skin after an intravenous administration of the virus. The same experimenter introduced fowl-pox virus into the wing veins of chicken after plucking some feathers from the right breast. The virus was found to have disappeared from the bloodstream within six or eight hours after the injection, and the blood thereafter remained non-infective until the eighth or tenth day, when lesions appeared at the spots from which feathers had been plucked; coincidently with the appearance of these lesions the blood again became infective, and remained so until the twenty-fifth or thirtieth day. If, while the virus was present in the blood, more feathers were plucked from another area, fresh lesions appeared in this area in from two to four days.

Moses, while studying infective myxoma in rabbits, found that if some of the hair of an animal were plucked out an intravenous injection of the virus was regularly followed by extensive and severe lesions in the depilated area.

Comparable effects have been obtained with the Rous chicken sarcoma. Rous, Murphy, and Tytler suggested that injury might be a factor in the infectivity of cell-free filtrates. They observed that such a filtrate of Rous sarcoma was more likely to produce a tumour if a little diatomaceous earth was added to it. Their attention was drawn also to the fact that in three instances after intravenous injection the resulting growth had its seat in a functioning ovary. Further evidence of the localisation of a virus in a functioning ovary has been put forward by Ikeda, who injected 4 or 5 c.c. of an aqueous extract of Rous sarcoma into the wing veins of young and prolific hens. These hens all died within a month. Every one had sarcoma at the site of injection, nearly half the total had tumours in the lung, and over 90 per cent. of them had ovarian tumours.

Pentimalli[1] performed laparotomy on fowls and then, with a red-hot cautery, made burns in the liver, spleen, kidney, and pectoral muscle. Two or three days later he injected some dried and powdered Rous sarcoma tissue suspended in Ringer's solution into a wing vein. This resulted frequently in the formation of tumours at the various seats of cauterisation and also at the site of intravenous puncture and in the laparotomy wound. In some later experiments[2] he varied the method, but came to much the same conclusion—namely, that the virus of Rous sarcoma, and the cellular elements also,

become selectively localised in tissues which have been damaged.

Suzue came to a similar conclusion, having observed that injections of kieselguhr, lycopodium, or powdered charcoal into any part of a chicken suffering from sarcoma led to the formation of a metastasis at the site where the foreign substance had been injected, whether this site was in the muscles, the liver, or the lung.

Mackenzie and Sturm injected various substances—embryonic tissue, scharlach-R, kieselguhr, and tar—into the pectoral muscles of fowls, and after varying intervals, followed this up by giving an intravenous injection of a filtrate of Rous sarcoma. Although the intervals between the two injections were rather long—three days or more—tumours appeared in several instances at the spot where the foreign substances had been introduced. They noticed that the earlier stages of the inflammatory reaction localised the tumour agent more regularly than the later stages.

Similar localising effects of inflammation were recorded by Fujinami and Hatano, who state that if simple granulation tissue or inflammatory spots are created in any portion of the body of a sarcoma-bearing chicken, the tumour-producing agent operates there and often results in the formation of a sarcoma.

Findlay[2] was able to produce local tumours by injecting histamine into the right pectoral muscle of fowls, after having given an intravenous or intraperitoneal dose of a cell-free filtrate of Rous sarcoma.

CHAPTER X

THE LOCALISATION OF CANCER

THE frequent association between irritation and cancer has been universally recognised. The precise nature of this association still awaits an explanation. The simple doctrine that chronic irritation causes cancer is found by the test of animal experimentation to need modification, for cancer is not readily or frequently produced in animals by persistent irritation alone. On the other hand, it has been accepted that the continued administration of arsenic by the mouth for a sufficiently long time is apt to result in the appearance of a cutaneous cancer which may be similar in every outward feature to the cutaneous cancer which has resulted from physical or chemical irritation applied directly to the surface of the skin. And this is true of animals and man. That a particular chemical substance can thus play a part in the process at once arouses the question as to whether irritation alone is indeed ever the cause of malignant disease.

In the years that have elapsed since Yamagiwa and Itchikawa first demonstrated that epithelioma could be produced in rabbits by applications of tar to the skin an increasing volume of evidence has accumulated to show that the nature of the tar used is the predominant factor in the causation of these experimental tumours, and that the chronic irritation which accompanies the applications is subsidiary and perhaps unessential. Furthermore, a spectroscopic method introduced at

the Cancer Hospital Research Institute by W. V. Mayneord led to the discovery of pure synthetic substances (1 : 2 : 5 : 6—dibenzanthracene and some allied compounds) which are carcinogenic (Kennaway and Hieger, Cook). To the writer these and associated observations made at the same Institute appear to undermine the hypothesis that chronic irritation plays the leading part in the causation of experimental tar cancer.

As a sequel to this line of thought arises the idea that the development of a spontaneous neoplasm may be due to a local concentration under the influence of chronic inflammation of some cancer-producing or cancer-favouring substance present in the blood. At any rate, it seems probable that if cancer-producing substances or their precursors do in fact find their way into the bloodstream and exist there in a colloid state, or even in the form of particles of rather more than colloidal dimensions, they will become concentrated in any tissues which are subjected to chronic irritation. So far as this idea rules, the chief part played by irritation in the production of a malignant tumour will be the concentration of a cancer-producing substance. This does not necessarily mean that chronic irritation plays no other part. It may be that often repeated or continued stimulation of cells prepares them in some manner for an indefinite period of multiplication with imperfect differentiation. On the other hand, it may be that, apart from its power to localise the cancer-producing substance, irritation in itself is inimical to cancerous growth. Yet a third possibility is that under the influence of sustained or repeated stimulation the cells directly affected may be enabled to manufacture

endogenously some material necessary for neoplastic development. These questions may be left out of the discussion at the moment except for the remark, in passing, that certain observations of Deelman, Murray,[1] Berenblum[1, 2] and others suggest that the less severe degrees of irritation may be more favourable to the production of tumours by painting with tar than are the higher degrees, and, in fact, that an excess of trauma may actually delay or prevent the development of cancer.

How slight may be the local stimulus required in certain instances for the localisation of matter from the bloodstream is illustrated by foot-and-mouth disease, the virus of which affects chiefly the mouth and the soles of the feet, where it becomes deposited as the result of the normal tissue disturbances, possibly vascular in character, which accompany standing and eating (p. 131).

The hypothesis that cancer formation is attributable to the localisation of a cancer-producing agent may be regarded as already established in certain cases. Clinical experience has made familiar the appearance of arsenical cancer in areas of skin where irritation has led already to a deposit of pigment; and unless we are prepared to believe that arsenic itself is not the cause of arsenical cancer, we must presume that the irritation which led to a deposit of pigment in an area where cancer subsequently appears is likely to have caused also a concentration there of arsenic.

To the rule that arsenical cancer is especially apt to appear in pigmented regions there are certain exceptions; for example, this form of malignant disease not infrequently affects the soles of the feet and the palms

of the hands where pigmentation is seldom conspicuous. In these situations the tumours arise chiefly in the parts most exposed to pressure; that is to say, on the heel and over the heads of the metatarsal bones—where the perforating ulcers of syphilis most often occur—and on the hypothenar eminence of the hand. These points are well shown in photographs which illustrate papers by Hamilton and Semon on arsenical cancer. Such apparent exceptions do not destroy the hypothesis of cancer causation which is here being advocated, for it is not necessary to assume that any particular stimulus will lead with equal influence to the localisation and retention of the pigment and of the arsenic. Moreover, there may be independent local factors which affect the outcome. Thus in the soles of the feet and palms of the hands there are present already conditions which lead to an increased growth of the cutaneous structures, and it may be that arsenic would have in such tissues an especially favourable medium for the production of cancer. At the same time there appears to be some reason why pigment does not become retained in large quantity in the soles and palms. The deficient pigmentation of these parts in the dark-skinned races is a matter of general knowledge.

The association of local pigmentation and cutaneous cancer is not confined to cases which arise from the ingestion of arsenic. Unna, Paul, and Molesworth have all remarked upon the frequent incidence of cancer due to excessive exposure to sunlight upon those regions of the skin which have become pigmented owing to such exposure. A similar sequence occurs in *Xeroderma pigmentosum*. Again, when an epithelioma develops in connection with an old scar, the growth appears

often at the margin of the scar, which is precisely where the localisation of natural pigment, of dyes given intravenously, of blood-borne viruses, and of syphilis, is most pronounced.

The idea is hard to avoid that a common factor is at work in these cases and that the increased permeability of the capillary walls, which is the essential cause of the localisation of pigment and other materials from the blood, may be also a cause of the cancer appearing in these same regions.

Reference has already been made in these pages to the effect of silicosis in localising coal-dust and tubercle bacilli in the lung, an effect which it seems should be attributed to the chronic inflammation brought about by the presence of the silica. That other foreign substances will similarly find a resting-place in the silicotic lung seems highly probable. The line of thought thus suggested may be followed further in connection with cancer of the lung. Rostoski, Saupe and Schmorl state that within the period of three years covered by their review, twenty-one of 154 miners employed at the Schneeberg mines died. Post-mortem examinations were made of thirteen of them, and cancer of the lung was found in every one. The average length of time during which these men had worked in the mines was twenty-five years, the shortest spell being ten years. In addition to these cases there were two others in which death occurred from cancer of the lung a long while after the individuals concerned had left the mines, the intervals being fifteen years in one case and twenty-two years in the other. In nine of these cases cancer had arisen in a bronchus and in six it had originated in lung tissue. The dust of these

mines contains silica, arsenic and radioactive matters. Radium emanation also is present in the air. The last-mentioned fact makes it difficult to claim these cases as due to the fixation in the lungs by inflammation of cancer-producing matters, although it seems likely that such fixation would occur in association with the silicosis. The same comments are applicable to the pulmonary cancer affecting the workers at the Joachimstal mines. Siki, however, who has inquired into this matter as it affects the miners of Joachimstal, does not find any evidence in the appearance or chemical analyses of the lungs to support the suggestion that the inhaled dust is the carcinogenic agent, which may be the radium emanation present in the air which these miners have been accustomed to respire.

That the mere irritation of silica by itself does not cause pulmonary cancer seems to be shown by general experience. That is to say, cancer of the lung is not a common cause of death even among miners affected with silicosis, provided that carcinogenic matters do not accompany the silica. Thus Schulte states that amongst 487 cases of silicosis occurring amongst the Ruhr miners there was not a single case of cancer. Apparently the sharp particles inhaled in the Ruhr are not different from those inhaled in the Schneeberg, hence the predisposing factors in the latter mines are more likely to be arsenical and radioactive particles, coupled, perhaps, with the inhalation of large amounts of radium emanation.

There is yet another standpoint from which this hypothesis of cancer causation may be discussed. Attention has already been drawn to the fact that in normal states colloidal and other matter in fine sus-

pension will become removed from the bloodstream by the phagocytic cells lining the sinusoids of the reticulo-endothelial organs, especially those of the liver and bone-marrow. When a focus of inflammation exists, the inflamed tissues will share with the reticulo-endothelial organs in localising these colloids and other particles. Some cancer-producing substances almost certainly become localised in the bone-marrow, and by associating this fact—assuming its correctness—with such knowledge as observation and experiment have provided, it must be regarded as probable that a localisation of these substances will be effected also by inflamed tissue. So far as the concentration of a cancer-producing substance in the bone-marrow is concerned, the following may be given in evidence. Martland and Humphries have described the conditions found post-mortem in two women who had died of sarcoma of bones and whose occupation had been the painting of watch dials with luminous paint. These women had been in the habit of pointing their brushes with their lips (Flinn), and hence had swallowed minute doses of the paint, which contained crystalline zinc sulphide with the addition of very small amounts of radium, mesothorium, and radiothorium. It appears that these materials after ingestion become stored in the reticulo-endothelium of the bone-marrow, where their first effect is to cause a rarefying osteitis combined with destruction of the blood-forming tissue. Bones so affected have but little resistance against infection, the jaws in particular being apt to become the seat of a spreading and intractable necrosis. If the patient survive these risks of necrosis and anæmia, after some years she is apt to suffer from osseous

sarcoma. Martland and Humphries state that the deposits of radioactive substances are evenly generalised over the entire skeleton, and emit their characteristic radiations for the rest of life and after the death of the patient. The sarcomata in these two subjects had occurred in the femur and scapula respectively. Haagensen has recorded two additional cases. In case (1) the patient had been employed in painting dials for fifteen months. In case (2) the patient had been occupied as a dial-painter from 1917 to 1921. In 1930 she was found to be suffering from a sarcoma of one femur. In a more recent paper Martland gives details of nine cases of sarcoma occurring in the bones of girls whose occupation had been the painting of watch dials with luminous paint.

Even more convincing, perhaps, than the above clinical evidence of the localisation of cancer-producing agents are the laboratory proofs that neoplastic viruses can be localised by irritation, so that if a slight lesion is caused in the skin or internal organs and the virus be given intravenously to a susceptible animal, tumours will arise at the site of the lesion (p. 135). Nor is an artificial trauma necessary for this sort of lesion; a physiological injury such as occurs in a functioning ovary appears to be sufficient to effect the localisation of a sarcoma-producing virus. Perhaps it will be easier to appreciate this pathological explanation of the local incidence of cancer if one remembers that the readiness with which colloids become concentrated in a particular part under the influence of stimulation depends largely upon the size of the particles. It will have been noticed in Chapter IX. that viruses are localised by irritations which would hardly be sufficient for the

attraction of bacteria. So it is that the virus of foot-and-mouth disease, which is said to be the smallest virus known, will become concentrated by the mere repetition of local pressures, a localisation which may be compared with the familiar occurrence of arsenical keratoses and cancers on the soles of the feet and palms of the hands.

Yet another argument can be used in favour of the view that cancer is produced by some special agent which becomes concentrated in a particular region owing to some pathological or physiological disturbance in that region. Thus Murray[2] has remarked that while cancer in this country, as shown by the Registrar-General's Reports, occurs with about equal frequency in men and women—due corrections having been made for age and total numbers—yet in women cancer affects the organs of sex in 40 per cent. of all cases, while cancer of the male sex organs represents only a little more than 4 per cent. of all cases in men. In other words, while the total liability to cancer is approximately equal in the two sexes, there is a wide discrepancy in its local incidence. It seems possible to account for such a variance of incidence between the two sexes, together with their equal liability, by assuming the presence in the body of some generally distributed causal agent of cancer which becomes concentrated in certain organs and tissues under the influence of those factors which are known to bring about a localisation of electronegative particles of colloidal size.

Equally striking is this kind of argument when we consider the mortality from malignant disease amongst the different nationalities and races. Murray[2] and Cramer[2, 3] have commented upon this subject. They

state that Holland, Switzerland, Japan, and England and Wales show an approximately equal incidence of cancer in males and females respectively. But whereas in England cancer of the sex organs is 40 per cent. of the total in women, in Holland it is only 20 per cent. Cancer of the breast is twice as common in English women as in Dutch women, while in Japan it is rare. Yet the total mortality from cancer among the women in these countries is almost the same. In Japan a low incidence of breast carcinoma is combined with an extremely high proportion of cancer of the uterus. In Holland, where cancer of the female sex organs is considerably less frequent than in Japan or England, malignant disease of the digestive tract is greater, so that the total incidence of cancer is equalised.

Sorsby has made an inquiry into the relative mortality from cancer among the Jews and Gentiles of several large towns, including London, Vienna, Amsterdam, Warsaw, Budapest, and Lodz. He has found that although the deaths from cancer vary considerably in number amongst these different Jewish communities, on the whole there is a fairly close approximation of the rate for the Jews to that of the non-Jews in each of the various cities studied. In fact, there is a closer agreement between the rates for Jews and non-Jews in each city than between those for the Jews dwelling in different towns. In other words, cancer among Jews, as compared with its occurrence among Gentiles, follows a geographical rather than a racial distribution. But the incidence of cancer for each organ of the body shows striking differences between Jews and non-Jews living in the same city. Cancer of the tongue and buccal cavity, penis, and uterus is notably less frequent in

Jews, while cancer of the intestinal tract, and perhaps of the ovaries too, is higher in Jews than in non-Jews. The greater part of these discrepancies can be explained, Sorsby believes, by the customs of Jewish life; though the heavier incidence of gastro-intestinal cancer is not, he remarks, to be explained on this basis.

If we were to accept irritation alone as the cause of cancer, the differences in its distribution in the body, as between men and women, or between various races and countries, might be accounted for by differences in circumstances and habits between the peoples concerned; but it would become difficult then to explain the striking similarity of the total numbers of cases. On the other hand, if we suppose the existence of a common and perhaps physiological underlying cause and attribute to irritation merely the localisation of this cause, it is not so difficult both to account for the similarity of the total incidence of cancer in the two sexes or in different races, and to understand the differences in its distribution.

Before dismissing the subject of the localisation of cancer-producing agents, there are one or two kindred problems which call for a brief discussion. Among these is the question as to whether a bone sarcoma can arise as the consequence of a single injury. It is not uncommon to meet with a case in which a tumour has arisen within a few months or less of the receipt of a blow. That the blow and the trauma may in some instances represent cause and effect is conceivable. Coley is a staunch believer in such a connection. "In sarcoma," he states, "especially in sarcoma of the long bones, there can be no longer the slightest question that a single local trauma in the form of a bruise, a

sprain, or a fracture, may be the direct exciting cause of the sarcoma." Among 360 cases of bone sarcoma seen by him, there was a definite history of antecedent trauma in 181, that is to say, in 50 per cent.; and in 133 (73 per cent.) of these 181 cases the tumour appeared within six months of the injury, and in 153 (84 per cent.) within the first year.

We may in this connection pursue an argument based on analogy with bacterial infections. Thus a child who happens to have *Staphylococcus aureus* circulating in the bloodstream receives a bruise on the tibia and thereafter suffers from acute osteomyelitis of the bruised bone. Experiment has shown that the *S. aureus* may become localised from the blood in an injured tissue, and the sequence of events in the example quoted is therefore logical. It is known also that the reticulo-endothelium of bone-marrow, apart from any injury, is one of the chief receptacles for any bacteria which happen to be present in the bloodstream, and it is almost certain therefore that osteomyelitis can come about in the absence of any localising trauma. Similarly, a footballer receives a severe kick on the tibia and within a few months is found to have a sarcoma at the site of the former injury. He himself has no doubt that the tumour is a direct result of the blow. He may be right. Seeing that several malignant tumours in animals are initiated by viruses and seeing also that these viruses readily become localised in injured tissues, we can hardly deny that a comparable process might take place in man; that is to say, that the reactive diapiresis of a local lesion due to trauma or any other cause might bring about the localisation of a neoplastic factor if this happens to exist and to

be present in the bloodstream. But it must be admitted also that, as in the case of acute osteomyelitis resulting from infection with the *S. aureus*, so with a sarcoma due to a suppositious minute agent, localisation in the reticulum of a bone would be likely to happen even in the absence of any injury. To pursue this matter further would be without profit, seeing that, in many of the cases in which trauma has been alleged to be the cause of a neoplasm, it is possible that the growth was already present, the real part played by the injury having been to make it evident.

The Localisation of Metastases.

Little evidence can be adduced in proof of the localisation of metastases by diapiresis, and the experimental work expended upon the subject is almost, though not quite, negligible.

Lubarsch, using mice with carcinomata that seldom if ever formed metastases in ordinary circumstances, tried the effect of (1) fracturing bones, and (2) puncturing the liver. No metastases occurred at the sites of fracture, but in two instances secondary carcinomata developed where the liver had been injured by puncture.

Pentimalli states that, while metastasis in the Rous chicken sarcoma can take place both by virus transport and by the entrance of cellular tumour elements into the bloodstream, in either case the metastases attack primarily those tissues in which proliferation has been inaugurated by trauma.

When cancer is growing in the human subject, detached cells or masses of cells may become separated from their point of origin and reach the general bloodstream either by way of the lymphatics or by direct

invasion of a bloodvessel. Arrived in the systemic blood they are carried to the lungs, in the capillaries of which they may lodge, establishing a secondary growth, or, passing through these capillaries, they then are apt to be arrested by the macrophage cells of the reticulo-endothelial sinusoids. Thus it comes about that the commonest sites of malignant metastasis, apart from the lymph nodes, are the lungs, liver, and bone-marrow.

Continuing to argue from analogy, it might be urged that, because colloids and bacteria which are removed from the bloodstream by the reticulo-endothelial organs are segregated also by inflamed tissue, it would seem likely that tumour cells which are known to lodge in the reticulo-endothelial organs would also become deposited in tissues which are inflamed.

As a matter of fact, in mankind the lodgment and growth of tumour cells in inflamed tissues is not nearly as frequent as such *a priori* reasoning might suggest, and if it ever does come about the occurrence must be uncommon. What evidence can be obtained is at best merely suggestive, and can carry but little weight.

The following case was originally reported by Cordinnier and Muller, and is quoted by Bourguina, from whose thesis the note has been abstracted:

A woman of forty-five, who was well educated and observant, had undergone excision of the rectum for carcinoma. Four years later she let drop on her foot a heavy weight which she was carrying. The blow caused a bruise and immediate severe pain, which continued for two months, at the end of which time a swelling was found at the site of the injury. She was not aware of any trouble in her foot before the

accident occurred. After another two months the foot was amputated, and was found to be the seat of an adeno-carcinoma which suggested a metastatic recurrence of the original tumour of the rectum. Six months later pulmonary metastases led to the patient's death. Lubarsch mentions a comparable instance.

In these cases, as in others of the same kind, it is impossible to know whether the injury caused the localisation of a metastatic deposit or whether it merely served to bring into evidence a secondary growth which was already present. And in such a state of uncertainty the matter must at present remain.

Intimately connected with this subject of the localisation of cancer by inflammation is the question as to whether a neoplasm finds its most favourable nidus in tissues which are normal or in those which are inflamed. Reference has already been made to the fact that in the artificial production of cancer mild degrees of inflammation appear to be more effective than are severer ones, and the suggestion was put forward that this might be due to the circumstance that gentle irritation is usually more efficient during an extended period of time in effecting localisation of colloids and certain other substances from the blood than is a stimulus of greater intensity. No conclusion can be drawn from this as to whether inflammation is or is not favourable to growth, once this has been started.

The few observations that have been made on the results of inserting tumour grafts into normal and inflamed tissues also leave the present matter in doubt. Levin states that the normal testicle of white rats proved resistant to grafts of Flexner Jobling carcinoma, only one such tumour transplant becoming established

among forty animals grafted. By inflaming the testicles prior to grafting, the proportion of takes was considerably increased.

Kubo, on the other hand, experimenting on fowls with Fujinami's myxosarcoma and chondroma, and using kieselguhr or turpentine as inflammatory agents, introducing them before, at the same time as, or after the implants, did not find in any case that the growth of the tumours was favourably influenced thereby. We must conclude, therefore, that so far as the small amount of evidence goes, any favourable influence which a mild inflammation may have upon the establishment and development of some cancer grafts is not generally displayed to all such implants regardless of their own peculiarities and of the particular circumstances of the experiment.

Jones and Rous found that, while peritoneal injection of a suspension of mouse tumour had little success in establishing successful implants, a preliminary injury of the peritoneum, caused by introducing kieselguhr, lycopodium or dead tumour fragments, led to a much greater number of successful takes. Even the insertion of a small, smooth glass rod destroyed the resistance of the peritoneum to grafting. This result does not necessarily mean that an inflamed tissue is the most favourable for cancer growth, though it does suggest that inflammation directly or indirectly favours the attachment of the graft to the host. That tumours can continue to grow in spite of inflammation, whether slight or severe, acute or chronic, is the general experience of mankind.

PART II

FACTORS IN LOCALISATION

CHAPTER XI

INCREASED PERMEABILITY OF CAPILLARY ENDOTHELIUM

In most departments of physiology complexities grow with advancing knowledge, and this is true of diapiresis and its consequences. The original notion that a dilated capillary presented apertures by which plasma and leucocytes could escape from the bloodstream, though attractive because of its simplicity, has had to give way to a collection of factors which, if still somewhat ill-defined, may yet be made the vehicles of a useful discussion. The agents which take part in the localisation of substances from the bloodstream fall naturally into three groups. In the first are those conditions which affect the permeability of the capillary endothelium; in the second are the forces responsible for the transport of substances from the bloodstream through the altered endothelium to their extravascular destination in the tissues; in the third are included those influences which lead to the retention of the substances which have escaped within the extravascular tissues at which they have arrived.

The general subject of permeation through the walls of the fine bloodvessels has been dealt with already in Chapter III., in connection with the formation of lymph, and only a few supplementary remarks will be necessary here. At the outset it has to be admitted that " permeability " of the endothelial cells is only a relative term, meaning merely that the cells are more readily

permeated than usual. It may be doubted if there is such a condition as complete and enduring impermeability. The flow of lymph itself involves the passage of colloid through endothelial cells. If a healthy white mouse be given repeated subcutaneous or intraperitoneal injections of the colloidal dye trypan blue, the animal will become almost generally stained, owing to a slow gradual transference of the dye into the perivascular tissues where it is taken up mainly by the macrophages. In spite of the slight leakage of colloid from the normal capillaries which such observations denote, the terms "permeability" and "impermeability" are too convenient to be discarded.

Further, when discussing the passage of colloids and fine particles through the capillary walls, it will be necessary to consider the words "permeability" and "permeation" as having somewhat different bearings. Without the former obviously there can be no permeation; but it does not equally follow that in the presence of permeability there will be free permeation, for this process is governed by incidental forces whose varying influences will regulate the speed and copiousness of the transportation.

The healthy bloodvessels are not all alike in their capacity to restrain the exodus of colloids from the blood. At one end of the scale are the capillaries of the central nervous system, which are among the least permeable, and at the other are the sinusoids of the liver. Whitney, regarding the protein content of lymph as an index of the permeability of the bloodvessels from which it was derived, from her own observations gave the following figures:

Source of Lymph.	*Protein Content.*
Cerebrospinal fluid	A mere trace.
Lower limb	2 to 4 per cent.
Liver	6 to 8 "

The work of Starling[1] on lymph formation first directed attention to the relatively high degree of permeability displayed by the sinusoids of the liver (p. 32). He referred to the fact that lymph from the liver is normally more concentrated than other lymph, and suggested that the simplest way of explaining this was to look on it as due to a difference in the permeability of the filtering medium. Although the degree of this permeability is not fixed and invariable, the hepatic sinusoids allow at most, if not at all times, an easy passage to colloids and even, perhaps, to suspended particles of larger size which happen to be present in the blood. Two peculiarities may be invoked, singly or together, to explain this important characteristic:

(*a*) Maximow[4] has maintained that the sinusoids of the liver have no endothelial lining. According to his view, these bloodvessels are lined only by macrophages whose origin is distinct from that of the true capillary endothelium. No effort is required to accept the easy permeability of vessels with such a lining. At the same time this argument should be applicable to the sinusoids of the bone-marrow and other organs of the reticulo-endothelial system as well as to the liver. Yet we have no evidence that it can be applied in so general a manner. McJunkin[3] combats Maximow's opinion as to the essential difference between the cells lining the ordinary capillaries and those of the hepatic sinusoids. By injecting casein or by crushing with forceps, McJunkin caused granulation tissue to form

in the livers of mice. At varying intervals afterward he gave 0·5 c.c. of Indian ink intravenously, and a few hours later killed the mice. He found a striking contrast as regards their carbon content between the capillaries of the granulation tissue and the sinusoids in uninvolved parts of the liver. Where the endothelium was not in contact with hepatic cells there was little phagocytosis. He concludes that in the absence of contact with living hepatic cells the endothelium becomes less phagocytic. In other words, he believes that the peculiarities of the sinusoidal endothelium are determined by its histological position, and that it is not a type of cell distinctive from that of normal capillary endothelium.

(*b*) Owing to the anatomical arrangement of the portal system the sinusoids of the liver are supplied with blood which has a low oxygen tension while containing a high concentration of carbon dioxide and other metabolites, and the permeability of the sinusoidal walls is perhaps wholly or partly associated with this fact.

Whatever the explanation may be, the liver provides an exception to the rule that capillary endothelium is normally an effective barrier against the free exodus of colloids from the blood.

The Nature of Endothelial Permeability.

The precise physical change which takes place when a vessel wall becomes permeable is not known. The first hypothesis—brought forward to explain the escape of white cells from the vessels—was to the effect that the capillary walls did not form a complete investment for the blood. Addison at one time thought that the

phenomenon of diapedesis corroborated "the views of those who entertain the opinion that the capillary distribution of the blood is situated in channels of the tissue, and not in vessels with a distinct membranous coat." Later Addison modified this opinion. The integrity of the capillary lining remained under suspicion, and there were those who suggested that, while in ordinary circumstances the vascular walls were complete, yet on the dilatation of a capillary the endothelial cells forming its wall became separated from each other here and there, so that clefts appeared between the edges of contiguous cells, through which clefts the white corpuscles and plasma could escape.

In recent years Krogh and Harrop have regarded the permeability of capillaries as due to the presence of "pores" in or between the endothelial cells, while Rinehart has boldly come forward to resuscitate in a modified form Addison's original hypothesis.

The orthodox though somewhat vague modern view of capillary permeability seems to have been first adumbrated by Lister, who remarked that the result of an irritation of the tissues which does not kill them is the production of a "condition bordering on loss of vitality, but quite distinct from it"—an observation which may sound indefinite, but which fairly represents our knowledge of the matter today. Lister noticed that dilatation of the capillaries was not an essential accompaniment of stasis, though in this condition the vessels may become quite empty of plasma and white corpuscles, owing to transudation and diapedesis. Since Lister's observations were published, other workers (Kulisch, Hoff, Tannenberg, Hirschfelder) have confirmed the fact that dilatation of the small bloodvessels is not

an essential element either of inflammation or of its accompanying transudation and diapedesis; it has been shown also that dilatation of the capillaries by itself does not lead to œdema or an increased flow of lymph. These and other observations make it difficult to accept any interpretation of the permeation of the capillary walls which is based on the presence of apertures between the cells. Indeed, several investigators (Florey, Lang, Stilwell, p. 8) have been able to perceive the actual transit of colloids and fine particles through the endothelial cytoplasm of capillaries rendered permeable by inflammation, a transit which has been likened by Fischer[1] to that of a drop of mercury which will pass through solidified gelatine under the influence of gravity without leaving any mark of its passage.

In this sense it is easy to imagine that when affected by inflammation or some other abnormal condition the capillary endothelium may offer less resistance than usual to the passage of colloids and particles from the blood, just as the resistance of gelatine to the transit of mercury may be lessened by thermal and other influences.

What the electrical and molecular changes may be which affect the permeability of endothelial cytoplasm is not known, and a detailed discussion of this topic at the present time would be fruitless. Life depends upon an ability to resist natural forces, and in this respect the relative impermeability of living cells represents a vital function. With death this ability disappears. There seems to be, as Lister remarked, an intermediate stage between life and death, in which the vital functions cease to be exercised to the full, and among these vital functions is a resistance to permeation.

Osterhout, working mainly with vegetable cells, observed that the increased permeability of injured living cells ran parallel with a simultaneous increase in their electrical conductivity. He was able to perceive, also, that an imperfect recovery after injury occasionally takes place, so that although to ordinary inspection the cells are completely normal, yet they continue to show an increase of electric conductivity together with an increased permeability. It would not be justifiable to assume without further study that the abnormal permeability in the neighbourhood of old scars in the human skin, for example, can be placed in the same category as these chronic effects of injury to seaweed; on the other hand, the analogy is too suggestive to be ignored. If imperfect recovery from injury cannot be invoked to explain the persistent permeability of the capillary endothelium in scar tissue, an alternative possibility is that the newly-formed vessels have failed to arrive at complete maturity.

Conditions which are accompanied by Increased Permeability of the Walls of Bloodvessels

(*a*) *Inflammation.*—The most familiar cause of an increased permeability of the vascular endothelium is inflammation, the main facts of diapedesis and the transudation of plasma in response to irritation having been recognised more than eighty years ago. Wherever inflammation occurs and from whatever cause, an increased permeability of the capillaries may be expected, with inflammatory œdema as a common result. Up to a point the mechanism of this process has been elucidated by Dale's work on histamine and by the later investigations of Lewis and others on the connection

between inflammation and the release of histamine-like substances; but the precise nature of the changes in the endothelial cytoplasm still remains obscure.

A special feature to be noted in connection with inflammatory œdema is that the increase of vascular permeability is confined strictly to the area which has been directly stimulated and does not involve the neighbouring vessels, which have undergone dilation under the influence of axon reflexes. Lister noticed that stasis caused by the application of mustard to a frog's foot involved only those vessels which were immediately subjacent to the irritant, while the neighbouring vessels, though dilated, showed no retardation of the blood flow. The fact can be neatly demonstrated by causing local irritation in an epilated area of the skin of a rabbit while a suitable dye is injected into an ear vein. As the writer has shown in some unpublished experiments, the dye will be found to mark out with an approximation to exactness the area of skin to which the irritating agent has been applied. This reaction to irritation is independent of any nervous impulses, and is an illustration of the fact that the essential element of inflammation is the direct response of those cells—and of those cells only—to which an irritant has been applied (Plate V., A and B).

(*b*) *Anoxæmia and Accumulation of Metabolites.*—On general physiological principles it appears possible that a deficiency of oxygen will cause an increased permeability of the capillary walls. The evidence that it does so, however, is somewhat scanty. One reason for this may lie in the circumstance that the presence of increased permeability is rendered evident as a rule only by increased permeation, and for the latter ad-

ditional factors are required; consequently when an increased permeation comes about it may be difficult to know which of the several conditions present is to be regarded as the primary cause. For example, in the case of venous obstruction, whether arising from cardiac disease or from blockage of an individual vessel, it may be easy to attribute the increased exudation which follows directly and entirely to the increased intracapillary pressure which obviously results, and to overlook the primary and essential but less obvious endothelial change which is present as an outcome perhaps of the anoxæmia. Moreover, a venous constriction sufficient to bring about anoxæmia in the tributary regions of the vein will be accompanied by an accumulation there of metabolites, and it may be these, either alone or in combination with anoxæmia, that cause the increased permeability of the capillary walls.

Clinical and other evidence indicates that cerebral tissue is peculiarly sensitive to a deprivation of oxygen; and it may be that the capillaries of the brain respond specifically to a lack of oxygen, and that their increased permeability under this influence cannot be regarded as an example of what happens generally in the vascular system.

Siengalewicz injected rabbits intravenously with trypan blue and then submitted them to inhalation of coal gas. After death the whole brain was found in these animals to have become stained blue, showing that the cerebral vessels had been rendered pervious to the dye by the poisoning, a result which, by inference, may be attributed to the anoxæmia which had been produced (pp. 29, 62).

Many of the dyes used for experimental injections in animals do not remain in perfect solution under usual circumstances, but after a while show by the microscope that some aggregation of the particles has occurred. When this change has taken place to a sufficient degree, the dye mass injected in an ordinary amount into the bloodstream of a rat causes dyspnœa from lodgment of the grosser dye particles in the capillaries of the lungs. In many cases a post-mortem examination after death due to such an occurrence reveals not only a deep coloration of the lungs from arrest of the dye in the capillaries, but a more or less pronounced staining of the brain; whereas properly made, fresh solutions of electronegative dyes do not cause dyspnœa when given intravenously, nor do they cause coloration of the lungs or brain. Such unpublished observations have been made on several occasions by the writer. The results appear confirmatory of the opinion that anoxæmia rapidly increases the permeability of the cerebral capillaries.

It is noteworthy in connection with this theme that the point of escape of colloids and cells from the capillaries of mammals corresponds with the site of the lowest tension of oxygen in these vessels. Thus it has been shown by Tannenberg that diapedesis in the rabbit's mesentery commences in the smallest veins and follows in the venous ends of the capillaries, coming about lastly in the arteriolar ends of the capillaries. Rous, Gilding, and Smith have similarly noticed that dyes injected into the bloodstream of rabbits and guinea-pigs escape into the tissues first through the venous ends of the capillaries. Furthermore, the sinusoids of the liver, where the oxygen tension of the

blood is especially low, are the most permeable portions of the vascular system, and it is reasonable to suspect that the low oxygen tension, together with the high content of metabolites, may have some causal relationship with the increased permeability.

Other experimental observations connected with the influence of anoxæmia and venous congestion in causing an increased permeability of the capillary walls have been quoted in a previous part of this work (*vide* Cohnheim, p. 29, and Lazarus-Barlow, p. 32, for experiments with anoxæmia, and Ranvier, p. 23, Emminghaus, p. 25, Cohnheim and Lichtheim, p. 26, also Bolton, p. 35, for results of venous congestion).

(*c*) *Variations of Hydrogen Ion Concentration.*—Slight changes of the hydrogen ion concentration of the blood have a pronounced effect upon the tone of muscle. Gaskell was the first to point this out. Some of his observations are as follows: (1) A frog's heart is perfused with a continuous stream of a 0·75 per cent. saline solution at a pressure of 20 cm. until it ceases to beat and remains in diastole. If, now, it is washed out with the same saline solution to which sodium hydrate has been added in a concentration of 1 : 20,000 the heart will begin to beat again, and when it stops it will remain in systole. (2) If the apex of a frog's heart be washed out with 0·75 per cent. saline solution it frequently remains relaxed without beating, in which case the application of dilute alkali will cause the heart apex to pass slowly from the position of extreme relaxation to that of full contraction. (3) Dilute acids, for example lactic acid, 1 : 20,000 in saline, have the reverse effect, and if by this means the ventricular beats have been very much lowered in force, an alkaline

solution will bring back the force of the beat to its original height. Gaskell explained that his chief object was "to draw attention as strongly as possible to the 'tonic' and 'atonic' condition of the whole vascular system produced by very dilute solutions of alkalies and acids respectively."

Cohnheim remarked that a strong flow of blood led to arterial contraction, while the arteries relaxed on being deprived of a blood flow. Dixon noted that lactic acid had the same effect on the frog's stomach that it had on plain muscle in other parts of the body and on the heart. Applied to the stomach in a concentration as small as 1 : 10,000, lactic acid checked the automatic contractions and within twenty minutes produced relaxation of the organ. A much stronger solution—*e.g.*, 1 : 500—caused rapid increase of tonus followed by gradual relaxation. These stronger solutions, however, when added to a stomach already undergoing relaxation as a result of an earlier application of a weaker solution of acid, merely increased the relaxation without producing an initial rise of tonus. Campbell, Douglas, Haldane, and Hobson were able to show that what the respiratory centre really responds to, when it responds to CO_2, is the balance of hydrogen-ion concentration in the blood. Fleisch demonstrated the vaso-dilator effect of carbonic acid in the frog and in warm-blooded animals, and further showed that the effect was not a specific one dependent upon the HCO_3, but that it was a response to a change in the hydrogen-ion concentration. It was not even necessary, in order to obtain vaso-dilatation, for the circulating medium to be rendered acid. A mere decrease of the alkalinity of the arterial blood from the normal to a pH of 7·1 was enough

to cause dilatation. The latent period for the vascular reaction he found to be between two and two and a half seconds.

Evans and Underhill, using the ileum of the rabbit and cat for their observations, also concluded that increases of hydrogen-ion concentration within the limits of viability caused relaxation of the tone of plain muscle. Excessive acidification led to death of the muscle in the relaxed state. In other experiments these workers used a guinea-pig's uterus. If the organ was placed in an alkaline bath with a pH of 8, small additions of acid caused a large contraction followed by rhythmic movements. But starting with a pH of 6, further acidification led to rapid relaxation without rhythmic contractions. "In the relaxed state," they say, "the tissue is quite insensitive to even large doses of oxytocic drugs such as histamine; the tissue is not dead, however, as can be demonstrated by changing the contents of the bath back again to a solution of about neutral reaction, when the response to the drug returns in a short time." After a while the tissue in the medium whose hydrogen-ion concentration has been changed from acid to neutral settles down at the altered pH and resumes its active response to histamine.

Hemingway transfused the hind limbs of cats and found the invariable response of the bloodvessels to an increased alkalinity of the perfusing fluid was a slight constriction, as shown by an increase of the peripheral resistance. Parallel with this increase of tone was an augmented responsiveness to adrenalin and pituitary extract. The reaction he found to be reversible—that is to say, alternate vascular relaxations and constrictions

were produced by alternating changes in the hydrogen-ion concentration of the perfusing fluid. Similar results were obtained with cat's uterus—that is to say, with an increased alkalinity of the fluid in which it was immersed the responses of the unstriped muscle to histamine and pituitary extract were augmented.

McSwiney and Newton immersed strips of smooth muscle from the stomach of rats, rabbits, and cats in fluids at different controlled reactions. All the experiments were started at a pH of 7·5, and within a certain range every change from this in an alkaline direction caused contraction and every change in an acid direction caused relaxation. The limits of pH between which this rule held were 5·9 to 11·5, and between these limits the changes were completely reversible.

McDowall prepared cats by preliminary injections of alkali, and then perfused their hind limbs *in situ* with fluid at a pH of 7·6, adding small amounts of acid to the perfusion fluid from time to time and noting the effects produced upon the resistance to outflow. At first each injection of acid invariably caused a brief dilatation, but eventually, after repeated doses, the vessels responded to further injections of acid by constriction. In unprepared animals, and also when the alkalinity of the perfused fluid was less than pH 7·4, the vessels did not recover from the dilatation caused by the temporary addition of acid, but the tone continued to fall until a stage was reached in which subsequent acid injections gave a constrictor effect. A strong dose of acid given initially caused a constriction followed by a considerable loss of tone (dilatation), but with repeated doses of acid given at intervals, a recovery of tone took place until it was higher than

before the first dose. Alkaline injections made when vessels are in a condition of acid tone at first caused dilatation only, but with repeated injections a constrictor effect was gradually produced.

McDowall's experiments seem to show that the reactions of the bloodvessels to the pH of the blood depend upon the conditions present at the time—that is to say, when there is no accumulation of acid in the tissues, an increased alkalinity of the blood causes vaso-constriction and an increased acidity causes vaso-dilatation. But when acid has accumulated in the tissues, then an increase of alkalinity of the blood causes vaso-dilatation, while acids have the opposite effect.

Put in another way, with a constant alkaline pH of the blood, an increase of acidity in the tissues leads to vaso-dilatation.

Rous (p. 196) has shown that the hydrogen-ion concentration of the tissues may show a wide range of variation in spite of the constancy of that of the blood. Schade, Neukirch, and Halbert (p. 197) and other observers have found that inflammation is accompanied by acidity, a fact that can be demonstrated by causing wheals on the skin of an animal which has been vitally stained with a suitable indicator, such as sodium phenol red, in which case the wheals after a while show a colour change denoting acidity (see Plate VIII., A).

Bazett and McGlone gave intravenous injections of phenolsulphonephthalein to decerebrate cats whose forelegs had been shaved. Normally this dye colours the skin a purplish-pink. Recent wounds, they observed, became purplish—that is to say, more alkaline. Cooling a limb increased the alkalinity of the skin by reduction of metabolism, while warming increased the

acidity, as also did arrest of the circulation. If the circulation of both legs were obstructed, and one leg was cooled while the other was warmed, the cooled leg showed no appreciable change of colour, while the warmed one showed a rapid increase of acidity. The development of acidity in the leg of a rat as a consequence of vascular obstruction is illustrated by Plate VIII., B.

Halliburton and McDowall, in discussing ciliary movement, state that it is arrested by deprivation of oxygen and restored by admission of oxygen. Dilute acids stop the movement and dilute alkalies restore it. If allowed to contract in a saline solution for a time cilia become fatigued and languid, owing to the accumulation of acid. When in this state their activity is restored by alkalies.

Lewis recorded the fact that if histamine is pricked into the skin while the circulation is arrested, whealing does not occur. Similarly, if a wheal has already formed as the result of stroking the skin in a case of factitious urticaria, or of pricking in histamine in a normal person, or from some other cause, whealing will not again occur in the same area until after an interval of time. Such irresponsiveness as regards whealing has followed a variety of initial inflammatory stimulations; and Lewis has noticed that while this state of irresponsiveness lasts the small vessels in the area concerned fail to respond to applications of adrenalin and pituitrin. In this condition the capillaries are atonic and dilated; and Lewis further notes that there are certain areas of the skin where the vessels sometimes remain continually in an atonic state and react feebly or not at all to applications of adrenalin, pituitrin, or histamine. He mentions in particular the

malar triangles and the darker areas of a mottled skin, and he notes that these same areas are particularly apt to become the seat of rashes due to measles and other ailments (p. 120). Renaut and Lewis have shown that these atonic areas in a reticulated skin are the areas which are least well supplied with arterial blood. Other clinicians (pp. 98, 100) have noticed that lesions due to syphilis, to various bacterial infections or to viruses, occur with a particularly heavy incidence in these atonic areas, which are, moreover, often the places where pigment becomes deposited in especial abundance (p. 88). Therefore it is safe to conclude that the atonic vessels of these areas are unduly permeable. That they do not respond to histamine by the production of whealing is probably to be attributed, not to a lack of permeability, but to a deficiency of those particular forces responsible for the abstraction of plasma from bloodvessels which have been rendered permeable. This long-lasting vascular atony appears to be of considerable significance in the localisation of disease, and will be discussed further on a subsequent page (p. 244).

To elucidate the preceding argument it may be summed up in this way. Any increase of the hydrogen-ion concentration in the extravascular tissues—the pH of the blood being relatively constant—will cause atony and vaso-dilatation of the small bloodvessels of the neighbourhood. These vessels will no longer respond by contraction to applications of pituitrin and adrenalin. They will be abnormally permeable to the colloids and suspended particles of the blood; but they will not respond to applications of histamine by the formation of wheals.

(*d*) *Circulating Poisons.*—No detailed argument is required to support the proposition that poisons brought into direct contact with the vascular endothelium will reduce its resistance to permeation. As physiological examples, the condition of the small bloodvessels which results from the intravenous injection of histamine or of an antigen into a sensitised animal may perhaps be quoted. The action of accumulated metabolites may also be mentioned. Some venoms and toxins, either by direct injury or by indirect influence, certainly render the capillaries incompetent, and it is fairly safe to assume that a variety of organic and inorganic chemical reagents may have a similar effect.

(*e*) *Defective Constitution of the Blood.*—Both experiment (*vide* Cohnheim and Lichtheim, p. 27) and clinical observation suggest that quantitative defects in the components of the blood may be enough to cause an increase of permeability of the capillary walls. There is one fallacy, however, to be avoided in drawing conclusions based on the presence or absence of œdema, for a reduction of the protein content of the blood by whatever means it may be produced will result in an excessive amount of water being held by the tissues; such an œdema cannot be accepted as reliable evidence of an excessive permeability of the vascular endothelium to colloids (*vide* Bennett, Dodds and Robertson).

(*f*) *Cellular Degeneration.*—Amyloid disease is a recognised example of this cause of increased endothelial permeability.

(*g*) *Physiological Activity*, if accompanied at all by an increase in the permeability of the capillaries concerned, is not a pronounced causal agent. It has been included in this category both because there seems

a priori to be a likelihood that a change in the resistance of the capillary endothelium might assist the interchanges necessary for continued active function, or for the storage of materials in preparation for activity, and also because experimental evidence, though admittedly somewhat slender, does suggest that a definite if slight increase of endothelial permeability precedes or accompanies the activity of an organ. The localisation of colloidal dyes in the areola of the mamma during its hypertrophy in pregnancy, and in the maternal part of the placenta, may be visible evidence of this. Rous and Smith have made some observations which have a bearing on the matter, and which will be mentioned again later (p. 194).

(*h*) *Immaturity*, and (*i*) *Imperfect Recovery after Injury*, are included in the list, because experiments with dyes and clinical observation both point to the probability that they are causes of capillary defect. The suggestions have practical bearings and appear to be novel, though confident statements are hardly justified at present. The field is a promising one for future investigation.

Some rather free speculations upon the subject will be made in the chapter on persistent endothelial permeability (p. 244).

(*j*) *Vaso-Dilatation.*—Dilatation of the capillaries has been included in this discussion of the causes of increased permeability of the vascular endothelium, although the part it plays, if any, is a minor one. That it has some influence is probable, for in spite of the fact that an explanation of vascular permeability based upon an anatomical porosity of the capillaries can be rejected, yet it is not unreasonable to suppose that a

dilated capillary with its thinned endothelium offering a large surface to the blood and tissues, will be more readily permeated than an undilated one. Certainly in the presence of inflammation, in which exudation and diapedesis are pronounced, vaso-dilatation usually is in evidence also. Lister clearly did not think dilatation essential for exudation, for he noticed that stasis followed applications of capsicum without any change in the calibre of the vessels concerned. Hirschfelder, while making observations upon the œdema produced in the conjunctiva and eyelids of rabbits by mustard oil, found that the instillation of a 10 per cent. solution of cocaine, so as to produce anæsthesia with vaso-constriction, inhibited the œdema, though not with any reliable regularity. Procaine and butyn, used in the same way, though causing anæsthesia, did not check the œdema at all. These two drugs do not produce vaso-constriction. He regarded the inhibiting effect of cocaine, therefore, as due to vaso-constriction, and not to its action upon the sensory nerves. At the same time he made the interesting observation that even when œdema was checked by the local application of cocaine the permeable condition of the capillary walls could still be demonstrated by the selective staining which followed an intravenous injection of trypan blue. He found, on the other hand, that conjunctival œdema produced by the application of mustard oil was increased by local vaso-dilatation, provided that this was not accompanied by an alteration of the general blood pressure. Thus a solution of sodium nitrate dropped into the eye led to an increase of the mustard oil œdema, but given intravenously, so as to lower the general blood pressure, it had the opposite

effect. From these observations, which illustrate the difficulty of placing their respective values upon several factors acting in unison, it seems that hydrostatic pressure and vaso-dilatation, though not causal agents of inflammatory œdema, may yet influence its volume.

Kulisch, experimenting on hairless African dogs, noticed that applications of cantharidin produced vesicles on a skin that was hardly reddened. Tannenberg, also, whilst making a study of inflammatory changes in a rabbit's mesentery, found that applications of a 1 per cent. solution of barium chloride caused contraction of the subjacent bloodvessels, including the capillaries, and at the same time brought about reversible stasis in these contracted vessels. In this case the arterial ends of the capillaries, during the period of stasis, became filled with pure plasma. The essential causes of stasis, he remarks, are those which produce an increase of the sedimentation capacity of the red blood corpuscles and a decrease of the surface tension of the plasma.

Hoff elicited the fact that, by stroking, a wheal could be raised in a patient with factitious urticaria even when the skin had been rendered pale by adrenalin. Moreover, if Congo red had been given intravenously beforehand, the dye appeared in the wheal. Vaso-dilatation, therefore, is not an essential factor in the causation of a local œdema; in other words, permeation is not prevented by vaso-constriction.

Landis,[2] studying the permeable mesenteric capillaries of frogs, observed that plasma began to pass readily through a capillary wall when the venous end was compressed, so as to raise the pressure within, although no alteration of the calibre of the vessel was

caused. By direct intracapillary measurements Landis[3] was able to correlate the changes of pressure and calibre in capillary vessels and the passage of dyes through their walls, and he came to the conclusion that the walls of dilated capillaries are not more pervious than those of constricted vessels, the rate of passage of a dye through the capillary wall being directly proportional to the difference between the intracapillary pressure and the osmotic pressure of the plasma proteins.

Rous and Smith, having studied the cutaneous circulation in *Rana clamitans*, say that after the injection of a poorly diffusible dye, it can be readily seen that some portions of the individual capillary mesh are not infrequently narrow, whereas others are broad. The escape of such dyes from the broad portion of the mesh often precedes that from the narrow, although the blood flows briskly in both. All in all, they say, the rule seemed to hold that, wherever the capillary was wider, other things being equal, more dye escaped through its wall.

The subject recalls an ingenious experiment by Florey,[1] who showed that by stretching a layer of gelatine, the rate of diffusion into it was increased.

Hare found that if histamine was pricked into the skin while the circulation was arrested, the puncture became surrounded by a purple area to which the subsequent wheal, arising on renewal of the circulation, exactly corresponded. This purple area afforded evidence that vascular dilatation was present in spite of the absence of whealing.

On the whole it would seem that, provided other conditions are favourable, capillary dilatation, considered by itself, may be regarded as an accessory

though subordinate factor in the occurrence of diapiresis. That it is not essential has been proved by several experimenters. A capillary dilatation alone cannot bring about diapiresis.

Summary.

The following conditions may cause an increased permeability of the endothelium of the small blood-vessels:

(1) Inflammation.

(2) Anoxæmia and accumulation of metabolites.

(3) Increase of hydrogen ion concentration in the blood or in the tissues.

(4) Poisons present in the circulating blood.

(5) Quantitative defects in the constitution of the blood (?).

(6) Certain cellular degenerations.

(7) Increased physiological activity (?).

(8) Immaturity of vascular endothelium (?).

(9) Imperfect recovery of endothelium following injury (?).

CHAPTER XII

THE TRANSPORT OF MATTER FROM THE BLOOD-STREAM TO THE TISSUES

ONCE it has become possible, owing to an increase of permeability of the capillary endothelium, for colloids and particulate matter to pass through the walls of the bloodvessels into the tissues, many forces previously held in check come into action and determine both the speed of the transport and the destination of the transported substances. The relative and absolute value of each one of these forces would form knowledge of great significance. At present any attempt to give a comprehensive account of them must fail, for the matter has been insufficiently studied and our knowledge is far from complete. The following remarks are intended to indicate the nature of the general problem rather than to assess the values of its component principles.

(*a*) *Increased Hydrostatic Pressure.*—There are different ways in which the blood-pressure within the capillaries can be raised experimentally. Only two will be considered here—namely, the production of plethora and venous obstruction.

It might be assumed that if the wall of a capillary were permeable to a colloid the velocity with which this traversed the endothelium in a direction from within outward would be increased by a rise of hydrostatic pressure within the vessel. If we were to accept a very slight degree of permeability as a normal condition of the

capillary endothelium, we should have to accept hydrostatic pressure as a contributory factor in the process of normal diapiresis. But so far as concerns the majority of healthy capillaries variations of hydrostatic pressure play a minor and almost negligible part. As will be seen later, the chief effect of a general increase of hydrostatic pressure within the blood vascular system is to cause an abnormally rapid escape of fluid through the permeable walls of the hepatic sinusoids.

Emminghaus observed that after division of the sciatic nerve the corresponding paw of a dog became warmer to the touch, and yet no increase of lymph flow resulted from the hyperæmia induced by the operation. If, now, the venous return of the limb was impeded, an increase of the escaping lymph followed. This increase he attributed to the rise of capillary pressure which the venous obstruction entailed. Since his experiments, many inquirers have recorded an increase of lymph flow as the result of impeding the venous bloodstream. As will be set forth later, however, other effects than a rise of capillary pressure follow venous congestion, and probably to these other effects the increased permeation of the capillaries is largely, if not chiefly, attributable.

The experiments of Cohnheim and Lichtheim on hydræmic plethora are of interest in this connection (p. 26). They injected quantities of saline solution amounting to as much as 90 per cent. of the animal's weight into dogs and cats without producing general anasarca. These large injections, while causing an enormous increase in the outflow from the thoracic duct, also brought about ascites and œdema of the alimentary canal on account of the natural permeability

of the hepatic sinusoids; they also produced a local œdema wherever any inflammatory focus was present, such as a healing wound, or a dermatitis brought about by heat or sunburn or chemical applications. Again, Cohnheim and Lichtheim found that ligation of the femoral vein was not followed by any œdema of the paw in ordinary circumstances; if, however, after the ligation, a large intravenous injection of saline was given, the limb whose main vein was obstructed at once became œdematous. Their results clearly show the secondary part played by increased hydrostatic pressure, the only œdematous tissues being (i.) the liver, whose bloodvessels normally are freely permeable; and (ii.) certain other structures, whose capillaries had become permeable as the consequence of inflammation or venous congestion.

Sherrington and Copeman noticed that a very small degree of venous obstruction increased to a detectable extent the specific gravity of the blood owing to an escape of fluid from the tributaries of the obstructed vessel. Thus an elastic band placed somewhat tightly round a rabbit's thigh for ten minutes caused a rise of the specific gravity of the blood from 1·046 to 1·055.

By experiments on cats Barcroft and Florey demonstrated clearly that ligation of the splenic vein in these animals was followed by an increased permeation of the small vessels of that organ. Cats were anæsthetised with chloralose, the spleen was exposed and blood samples were taken from the splenic pulp and from the peripheral vessels, for comparison. The spleen, having been made to contract by faradic stimulation so as to expel the blood, was tied at its middle with a thick ligature so as to divide it into two parts, and the main

vein from one of these parts was ligated. In five such experiments a considerable concentration of hæmoglobin appeared in the blood of the congested half, and it was further shown that this concentration was due to a removal of fluid which drained away through the lymphatic vessels.

Heidenhain's work on the formation of lymph is of interest in this connection. He noted that the quantity of lymph escaping from the thoracic duct after ligation of the portal vein was much increased, and he believed that the increase was to be explained by an increased filtration in consequence of the raised pressure in the capillaries distal to the ligature. But he also found that if, after tying the portal vein, the aorta was occluded also at a level above the diaphragm, the flow of lymph continued in spite of a fall of the blood-pressure to nil. Hydrostatic pressure could no longer be the controlling agent in these circumstances, and Heidenhain was led to the conclusion that this experiment pointed to the secretion as against the filtration of lymph. Today it may be attributed with more confidence to anoxæmia and poisoning by metabolites combined with certain other forces, to be considered on a subsequent page.

Starling noticed that active hyperæmia brought about no augmentation of the normal lymph flow, whereas venous obstruction had that effect. Blockage of the inferior vena cava above the diaphragm led to a greater output from the thoracic duct, the amount rising from 3 c.c. in ten minutes to 25 c.c. in ten minutes, and this rise in quantity was accompanied by a rise in the percentage of solids from 4·8 to 6·6 per cent. Starling stated that this experiment, which proved that an increased permeation followed venous obstruction,

could always be repeated with success. He regarded the result as due to a rise in the capillary pressure, and demonstrated the fact that most of the lymph was derived from the liver. At the same time in varying conditions there were differences to be noted in the rates of lymph flow and of the percentages of solids contained in the lymph, for which, as Starling pointed out, the simplest explanation was that they were due to differences in the permeability of the filtering medium.

Bolton (p. 35), repeating these experiments in a critical spirit and with some modifications, concluded that in the presence of venous obstruction the nutrition of the capillary wall was affected, and that its increased permeability was the direct consequence of this nutritional defect, and was not attributable entirely or primarily to a rise of capillary pressure. He noted that the increased intravascular pressure following blockage of the vena cava above the diaphragm resulted not only in ascites and œdema of the retroperitoneal tissues, but also of the tissues surrounding the operation wound, although no œdema appeared in uninjured subcutaneous tissues. These results are comparable with those obtained by Cohnheim and Lichtheim following artificial plethora, and like these they show that permeability of the vessel wall is the basic requirement for exudation, and that whenever, but not until, this permeability is present hydrostatic pressure will become a material factor in the process.

Magnus studied the effects of an artificial hydræmic plethora and observed that, although in healthy living rabbits the blood could be much diluted without causing subcutaneous œdema, in animals killed by poisoning

with arsenic, chloroform, chloral hydrate or ether, such dilution caused extensive general œdema.

The immediate effect of artificial hydræmic plethora is a great rise of pressure in the vena cava and the portal vein, so that there is a large rise of pressure also in the capillaries from which these venous trunks are supplied with blood.

Bayliss and Starling, experimenting with dogs, made careful measurements of the pressures within the aorta, portal vein, and vena cava following the intravascular injection of large amounts of saline. A considerable rise took place in the aortic pressure, and very large increases were found in the pressures within the portal vein and the vena cava. They concluded that a great rise of pressure must have been present in the capillaries.

The effect of a lowered blood-pressure upon inflammatory exudation was the subject of an experimental inquiry by Hirschfelder (p. 176), who found that a preliminary ligation of the carotid artery prevented to some extent the œdema of the conjunctiva and eyelids which ordinarily follows the application of mustard oil to a rabbit's eye. Further, the œdema could be lessened by giving an intravenous injection of a solution of sodium nitrite so as to lower the general blood-pressure.

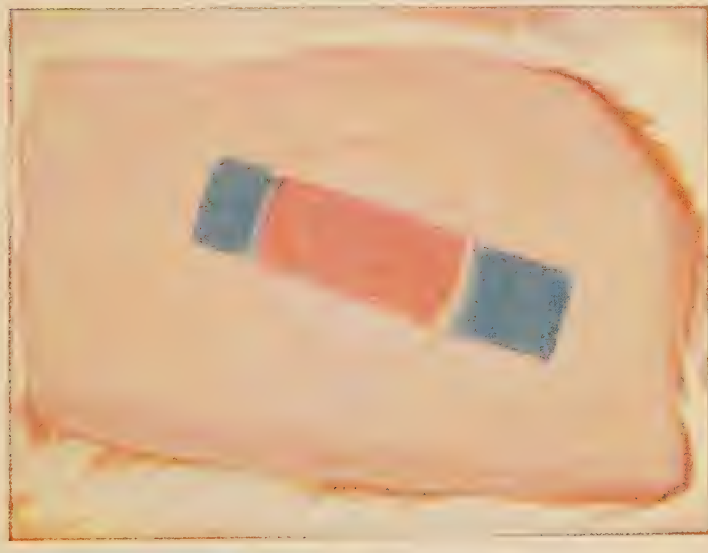
From the foregoing arguments it can be concluded that when the capillary walls are permeable, hydrostatic pressure may be a minor factor in transudation. In the absence of an increased permeability hydrostatic pressure has little or no influence. Venous obstruction acts in two ways: first by increasing the permeability of the vessel walls—an effect which may be explained by

the presence of a relative anoxæmia together with an increased concentration of metabolites in the blood distal to the impediment; and secondly, by the rise of intracapillary pressure which the venous obstruction entails. Clinical experience has taught that moderate venous obstruction, though not quite negligible, is by itself a feeble influence in the causation of œdema; were it otherwise tight garters could not be worn (p. 20). With a more pronounced hindrance to the return of blood, the effect upon transudation becomes obvious.

These conclusions are in no way incompatible with the results obtained by Lewis in his experiments upon the production of inflammatory œdema in man and the influence of hydrostatic pressure thereon. By stroking the skin of susceptible people and by pricking histamine into the skin of others and applying an armlet at once over the stimulated area, Lewis was able to show that the wheals which resulted would still develop even against a pressure of 50 mm. of mercury. He has shown, on the other hand, that an increase of venous pressure, produced by an armlet on the upper arm so as to compress the veins, neither expedites the appearance of a wheal in response to appropriate stimulation of the skin distal to the armlet, nor exaggerates its prominence. On the contrary, the wheal will be smaller than a control one developing from a similar stimulus on the other arm which has not been constricted. He further proved that the difference is not due to masking by a light general œdema of the congested limb. The higher the venous pressure, he states, the smaller are the wheals. Lewis concludes from these experiments that whealing is not due to a

Localisation of dye in an artificial wheal.

A. Isamine blue was injected into a rabbit's ear vein. Immediately afterward a B shaped wheal was raised in an epilated area of the animal's back. The dye has become localised with exactitude the irritated area of the skin.



Effect of suction on the localisation of dye.

B. A rectangular wheal was made in a rabbit, and a suction cup then applied with a negative pressure of 4 cm Hg. Isamine blue, injected into a ear vein, has become localised in the part of the wheal outside the cup but not

mere difference of hydrostatic pressure within and without the finer vessels.

Simple suction, however intense, never produces wheals, even on susceptible skins; moreover, applied over a developing wheal, suction, like increased venous pressure, prevents this wheal from forming fully.

Hare pricked horse serum and pollen extract into the skin of sensitive patients and compared the wheals with those produced by histamine. He observed that whealing was delayed with increasing venous obstruction, and that wheals almost ceased to form when the compression equalled the diastolic pressure of the blood. With complete vascular obstruction no wheals formed, though they arose as soon as the circulation was restored. External pressures of 25 to 35 mm. of mercury prevented wheal formation.

In experiments of my own (p. 69) I found that when suction equal to or greater than 4 cm. of mercury was applied by means of a Bier's cup to the shaved skin of a rabbit which had just received an intravenous injection of isamine blue, the dye did not become localised in the congested skin within the cup. Furthermore, if just before injecting the dye some chloroform were applied to the rabbit's skin so as to cause a wheal, cupping applied to this area with a suction pressure of 4 cm. of mercury or more entirely prevented any appearance of the dye in that part of the wheal subjected to cupping, though uncupped parts of the same wheal became stained a bright blue. Suction pressures of less than 4 cm. of mercury were not tested (Plate V., B).

These results of congestion at first sight appear anomalous and contradictory of the preceding con-

clusions as to the effects of increased hydrostatic blood-pressure and venous obstruction.

Cohnheim, who had noticed that a flow of lymph did not result from the application of suction, remarked that congestion by cupping differs entirely from active hyperæmia, because the partial vacuum within the cup exerts its exhaustive action in all directions, so that blood will enter the cupped region not merely from the arteries, but also from the neighbouring capillaries and veins; and while in a hyperæmic part a large quantity of blood flows with increased rapidity through the vessels, at the seat of cupping a complete standstill of the blood develops.

Lewis adopts a similar explanation. The wheals, he says, are lessened when such pressures are applied to the veins as are calculated to produce a diminution of the blood flow. The higher the venous pressure used, the smaller the wheals; when pressures approach closely or go beyond systolic arterial pressure, wheals are never formed.

Landis[2] has made some direct measurements of pressures in the capillaries of a frog's mesentery by means of micro-pipettes. He remarks that plasma will pass through the wall of a capillary whose venous end is compressed so as to raise the intracapillary pressure. He thinks that oxygen lack cannot be the explanation of this leakage because the vessel under observation was in the exposed mesentery, and he finds a full explanation in the raised pressure. However, it has to be remembered that a frog's capillaries are normally permeable to blood plasma; and consequently the rate of transit of this plasma through the capillary wall will always show a direct response to alterations of the

hydrostatic pressure within the capillary. Interesting as they are, Landis's conclusions can be applied to human physiology only in circumstances which are accompanied by a concomitant permeability of the walls of the finer bloodvessels.

Further, Landis was able to observe that the average pressure in a mesenteric capillary of a frog at the arterial end was above that of the colloids of frog's plasma, whereas at the venous end it was below. Therefore if hydrostatic pressure played a leading part in the transit of colloidal dyes they would, presumably, escape first from the arterial end of the capillary. This he found to be the case, although the result is not in accord with the observations of Tannenberg on rabbits (p. 40), who found that in diapedesis the escape of leucocytes occurred first in the venules, then in the venous ends of the capillaries, and lastly in the arterioles; or with those of Rous, Gilding, and Smith (p. 40), who observed that dyes escaped from the venous end of a capillary before escaping from the arterial end. Rous and Smith have repeated the experiments of Landis without substantiating his results, which they conclude were the consequence of damage inflicted on the capillary endothelium.

It is certain that transudation may take place even though the hydrostatic pressure within the vessels is abnormally low or that of the tissues surrounding them is abnormally high. Evidence of the former proposition is provided by the condition of general traumatic shock, in which, as Sherrington and Copeman pointed out, there is a widespread transference of plasma from the vessels into the surrounding tissues. In shock the arterial and venous pressures are both

reduced, and the capillary pressure therefore must be reduced also.

Dale, Laidlaw, and Richards, when studying histamine shocks in cats, estimated that the blood volume was reduced by as much as 40 per cent. owing to the passage of plasma from the vessels into the tissues. The depletion was accompanied by a general lowering of the blood-pressure. In their own words:

"If the chest is opened, under continued artificial respiration, it is seen that the heart is indeed beating strongly and regularly, but that its chambers contain remarkably little blood. Compression of the ventricles between the fingers causes scarcely a movement of the mercury in the arterial manometer. The venæ cavæ in the thorax are seen to be flaccid and half empty, and when the abdomen is opened it is seen that the abdominal cava is poorly filled with blood, while the portal vein is flat and almost empty. The arteries, both large and small, similarly contain but little blood, and appear to be constricted rather than dilated. The liver is moderately pale and certainly not distended with blood, and the same is true of the spleen."

On the other hand, exudation will take place in spite of a high extravascular pressure. Lewis and also Hare have shown that wheals will form on the skin in spite of external pressures, applied by an armlet, ranging from 25 to 35 mm. Hg. (Hare) and 30 to 50 mm. Hg. (Lewis).

The high tension that may exist in an abscess cavity is a pathological example of exudation against high pressure.

In summing up it may be asserted that while an increase of hydrostatic pressure by itself does not cause transudation of colloids through the walls of vessels

which normally are impermeable to these substances, yet in the presence of an increase of permeability a rise of hydrostatic pressure within the capillaries may affect the copiousness of the outpouring. In the liver, whose sinusoids constantly permit the passage of colloids, a rise of hydrostatic pressure within the portal capillaries will result at once in an increased outpouring of lymph. While making these statements, it has to be admitted that, given the presence of endothelial permeability, the difference between the hydrostatic pressures within and without the bloodvessels is only one of the less important factors by which the process of diapiresis is regulated.

In the presence of an abscess, transudation will occur in spite of the fact that the hydrostatic pressure within the abscess is much above that within the small bloodvessels.

(*b*) *Hyperæmia.*—Unfortunately the word hyperæmia often has been misapplied to passive congestion caused by impediment of the venous return; it has been used also as though it were synonymous with inflammation. Throughout this book the term denotes only a condition in which there is a dilatation of the arterioles, capillaries, and venules, with an increased flow of blood through them; and unless inflammation is mentioned, it must be assumed to be absent. For, striking though it may be as an accompaniment of inflammation, hyperæmia occurs apart from that condition and is accompanied by a different range of phenomena. Lister drew a clear distinction between the two states. He remarked that with the application of mustard to the web of a frog's foot, dilatation of the arteries took place over a wider area than that to which the mustard

had been applied, whereas stasis was strictly limited to the region of the application. Dilatation of the arteries in response to irritants he regarded as a functional phenomenon developed indirectly through the medium of the nervous system, whereas stasis was the result of the direct operation of the irritating agent upon the tissues.

Hyperæmia, as used in the sense thus defined, has by itself but little effect upon the output of lymph. The capillary pressure being raised, it might have been expected that there would be an increase of that small amount of capillary permeation that seems to be remittently or continuously in progress in normal circumstances. Experiments have shown, however, that little, if any, detectable increase accompanies hyperæmia. The blood in hyperæmia is well oxygenated and relatively free from metabolites; and perhaps these circumstances are sufficient to counteract the effects of the raised capillary pressure and copious supply of blood.

Paschutin was unable to find any additional lymph flow resulting from an active hyperæmia caused by section of the brachial plexus, even when the spinal cord was stimulated in order to raise the arterial pressure.

Emminghaus divided the sciatic nerve of a dog and noticed that the paw of that leg became hotter than its fellow. Nevertheless there was no increase of lymph flow; nor did any increase occur when the peripheral end of the cut sciatic nerve was irritated.

Janowsky also found no enhancement of the lymph output from the hind limb of a dog rendered hyperæmic by division of the sciatic nerve, but if both hind

feet of a dog were inflamed by injections of turpentine and then one sciatic nerve was cut through, the lymph flow was nearly doubled on the side of the nerve section (p. 29).

Rogowicz catheterised lymph vessels in both hind legs of a dog and then divided the sciatic nerve on the right side. An increased lymph flow occurred on the side of the severed nerve (2·95 c.c. as compared with 1 c.c., in thirty-five minutes), but this increase was temporary only, although the temperature of the limb whose sciatic nerve had been cut remained considerably higher than that of its fellow.

Two difficulties arise in the course of experiments such as these. One is the difficulty of ensuring a local hyperæmia which is unaccompanied by any injury; and the other is the fact that almost no lymph will flow from a normal limb in the absence of massage or movement.

In the experiments of Rogowicz mentioned above there was trauma of a rather pronounced degree owing to the catheterisation of the lymphatic vessels, and this trauma somewhat vitiates the experiment.

From the foregoing observations it may be gathered that hyperæmia unaccompanied by any condition causing an increase of endothelial permeability will not of itself bring about any considerable augmentation in the output of lymph. At the same time it seems that an artificially produced hyperæmia is capable of adding considerably to the volume of lymph derived in a given time from a limb the permeability of whose vessels has been increased by some other cause. This conclusion is in accord with an observation made by Lewis in connection with the formation of artificial

wheals in the skin. He found that a considerable arteriolar dilatation, though possibly unessential to whealing, is essential to quick whealing.

On the other hand, as remarked before, in cases of profound shock and in certain local conditions involving a diminution of blood flow, extreme degrees of exudation may come about from the capillaries. In capillary stasis, for example, the vessel concerned becomes quite free from plasma and white corpuscles owing to their escape through the endothelium, the red corpuscles which are left in the vessel after this exodus being so closely packed together as to appear under the microscope as a translucent homogeneous mass. And yet this condition may presently become reversed and, as Florey[2] has remarked, without any change occurring in the calibre of the vessel under scrutiny, the impacted red cells will gradually break away into the contiguous moving stream of blood; and normal circulation will be resumed.

These facts afford sufficient proof that hyperæmia is not an essential factor in capillary permeation, even though in certain circumstances it appears to be a favouring one.

Functional requirements might be expected to demand an increase of capillary permeation in addition to the increased blood flow which normally accompanies local activity. Clear evidence on this point is wanting, though it may be recalled that Rous and Smith, studying the cutaneous bloodvessels of the frog, frequently found "a distinctively great permeability of the capillaries which encircle the skin glands . . . under conditions which suggested that functional activity might be its determining cause." Beyond being

suggestive, this observation cannot yet be regarded as throwing light on the problem as it affects mankind.

From the foregoing discussion three conclusions can be drawn, as follows:

(1) Hyperæmia alone has little effect on the output of lymph.

(2) In the presence of increased permeability of the capillary walls due to inflammation hyperæmia tends to accelerate the transudation of lymph.

(3) A profound degree of transudation may occur in the absence of any hyperæmia.

(*c*) *Changes of Hydrogen-Ion Concentration.*—Fischer[1] was of opinion that the chief factor in the causation of inflammatory œdema was the increased imbibition of water by the colloids of the injured tissue, due to the presence of acids formed as a result of the injury. He believed that the reason why dead tissues swell when placed in water is that they become acid, and he showed that "wheals" could be produced on a gelatine plate by pricking it with a needle which had been dipped in formic acid and then pouring a little water on to the plate. These artificial wheals resembled in shape and rate of development the wheal resulting from a fleabite or from pricking formic acid into the skin. Objection can be raised against applying these observations as an explanation of inflammatory œdema. That increased acidity will lead to increased imbibition of water by the colloids of the living body may be true, and must be remembered, particularly when conclusions are being drawn from clinical observations, but inflammation causes not only an increase of the amount of lymph produced, but the lymph itself becomes richer in protein. Moreover, Lewis has shown that the fluid of

wheals contains all the proteins of the blood plasma. Their presence can hardly be attributed to the imbibition of water consequent upon an increase of local acidity.

Rous has demonstrated, by the use of chemical indicators, that the hydrogen-ion concentration of the living tissues may show a wide variation in spite of the relative constancy of that of the blood. He observed in mice vitally stained with phthalein dyes that while blood plasma from the right heart showed a colour which indicated a pH of 7·38, the most alkaline of the tissues—connective tissue—gave the colour corresponding to a pH of 7·2 or less. Most of the other tissues were apparently at least as acid as 6·6. Rous and Drury performed the following experiments: A rat was shaved and given enough phenol red intravenously to produce in the skin a general pink tinge which corresponded with a pH of 7·4 or a little less. Epinephrine now injected subcutaneously so as to cause a patch of vasoconstriction resulted in a local change of colour from pink to yellow corresponding with an increase of acidity, presumably owing to the increased concentration of CO_2. On everting this skin and exposing it to the air it became pink again through the rapid escape of CO_2. Bleeding an animal which had been dyed with phenol red caused the colour of the skin to become yellow, and so also did the administration of a hypertonic solution of sodium carbonate given by the mouth, although at the same time this increased the alkalinity of the blood.

Following the work of Rous and his co-workers, the writer has stained animals with sodium phenol red and then caused wheals to appear on the skin by applying chloroform. These wheals at first are similar in

colour to the surrounding skin, which is of an alizarin crimson tint, but after a short while they become less alkaline and so take on a vermilion (rat) or orange-scarlet tint (frog), and remain so coloured until they fade.

These are the details of such an experiment. An area of skin on the back of an old black-and-white rat weighing 300 grammes was epilated. A week later the animal was anæsthetised and a strand of soft wick was fastened round the left lower limb just above the ankle so as to impede the return of blood. A strip of filter-paper wet with chloroform was applied for thirty seconds to the epilated dorsal skin. Immediately after this 7 c.c. of a 2 per cent. solution of sodium phenol red were injected into the right femoral vein. A snip was then made with scissors in the epilated skin. The injection of dye caused a uniform pinkish-crimson colouring of the skin, including the wheal. An hour later the wheal resulting from the application of chloroform had a vermilion tint which showed it to be distinctly more acid than the rest of the skin. No difference was seen between the hue of the edges of the small skin incision and the normal skin. The limb submitted to venous congestion was of a yellowish-red, in striking contrast with the colour of the normal limb, though an inflamed toe did not show much change from the normal colour (Plate VIII., B).

Several investigators have found that inflammation is accompanied by acidity. Schade, Neukirch, and Halbert, testing inflamed tissues *in vivo* by means of a subcutaneous electrode, found a pronounced increase of hydrogen-ion concentration, the grade of which seemed to have some relationship with the degree of inflammation, as the appended table shows:

Condition.	*Hydrogen-Ion Concentration.*
Centre of a furuncle	5·96
Acute axillary abscess	5·98
Phlegmonous abscess	6·05
Acute empyema	6·24
Impetigo bullosa	6·44
Chronic empyema	6·57
Coxitis tuberculosa	6·58
″ ″	6·81
Cold abscess	6·91
″ ″	7·00
Pleural exudate (? T.B.)	7·00
Tuberculous ascites	7·03
Pleural exudate (carcinomatous)	7·09
Ascites due to morbus cordis	7·21
Average of five healthy tissues	7·21

(*d*) *Osmotic Pressure.*—Starling remarked that since the final result of metabolism in the animal cell is disintegration, a breaking-down of large, complex, unstable molecules of high potential energy into a great number of small, simple, stable molecules of small potential energy, the total output of an animal cell must have a higher osmotic pressure than the total income, so that all the metabolic changes in the tissues would tend to increase the osmotic pressure of the lymph with which they are bathed, and thus produce a current of fluid directed from the bloodstream toward the extravascular tissues.

The actual difference between the osmotic pressure of the blood and that of the lymph may be greatly diminished in certain pathological conditions, as reported by Bennett, Dodds, and Robertson. They found that if from any cause the plasma proteins fell to about 4 per cent., œdema resulted. Among the causes of such a large loss of albumen they included ascites and chronic gastric ulcer. One might suppose that in such cases the œdema resulting from inflammation would be increased.

Regarding the transference of colloids in the normal individual, L. Loeb has remarked, the filtration pressure required is always present so that transudation will occur at once, provided that other factors, including the condition of the capillary wall, are favourable. There can be little doubt that as soon as the vascular endothelium has ceased to be impermeable to colloids, differences of osmotic pressure will take a larger part in regulating the exchanges between the bloodstream and the tissues outside. At the same time it will have to be admitted that osmosis at the most is only one of several secondary agents that play a part in such circumstances.

(*e*) *Electrophoresis.* — Unfortunately, beyond the present point the discussion of the forces which regulate diapedesis and exudation becomes increasingly difficult. The position seems to be this: as soon as the endothelium of the small vessels has become permeable from any cause, several forces hitherto held in abeyance now come into effective action, and under their influence colloids and suspended matter, including blood cells, will be transferred from the interior to the exterior of the affected vessels. Hydrostatic and osmotic pressures obviously do not play the leading parts in these transferences. In the course of inflammatory stasis, for example, although the intracapillary pressure may be below the normal, there is a complete transfer of all the contained plasma and white blood corpuscles from within the small vessels to the extravascular tissue spaces. In general traumatic shock, also, there is a copious escape of plasma from the capillaries in spite of a lowered hydrostatic pressure within the bloodvessels. Moreover, diapedesis, which

Cohnheim showed to be independent of any motile power possessed by the leucocytes, cannot be explained in terms of hydrostatic pressure, osmosis or diffusion.

From the time of Du Bois Reymond, electrical disturbances have been known to accompany trauma of the living tissues, the injured surface becoming electro-positive in respect to the uninjured. Schwyzer seems to have been the first to suggest that differences of electrical potential consequent upon injury might be responsible for some of the phenomena of inflammation; and in the last few years much useful inquiry has been made into the possibilities contained in this suggestion. There is now reason to believe that the diapiresis of colloids, free inorganic particles and organised bodies, including bacteria and leucocytes, is due mainly to electrical agency. These migrating matters all carry negative charges of electricity while in the blood, and will therefore be attracted towards any tissue which is relatively positive.

Abramson,[1, 2] who has carried out a careful investigation of this matter, finds that the magnitude of the fall of potential between injured and uninjured tissues is sufficient to cause the migration of cells. The currents passing between injured and uninjured tissues, he points out, must have their origin in (1) membrane potentials produced by intervening phases, or in (2) diffusion potentials based on differences of "ionic molarities." Membrane potentials, he says, usually are of the order of 0·02 to 0·1 volt, but a particle migrating cataphoretically would be out of range of this main drop of potential except while actually passing through the phase—that is to say, in the case we are considering, the wall of the capillary. Owing to the tenuity of the

capillary endothelium the relatively high differences of potential on its inner and outer surfaces may be ample to effect diapedesis. From diffusion potentials, he says, only slight electromotive forces are to be expected—not greater than 0·01 volt.

Though conductors, the tissue fluids are not good conductors; it may therefore, he thinks, be assumed that even if the total drop in potential is not uniform, a portion of it, sufficiently great to influence the migration of a charged particle, is present. The potentials measured between a cut surface and an uninjured surface vary between 0·030 and 0·100 volt. If diffusion potentials only are present, and if the potential difference is only 5 millivolts, with a distance of 0·05 mm., the potential difference per cm. is 1 volt—" a force sufficient to bring a leucocyte moving in plasma at the rate of 5 μ per volt per cm. per sec. to the point of injury in two minutes."

The appended table, which is taken from one of Abramson's[2] papers, gives an idea of the forces which may be available for electrophoresis *in vivo*:

Distance between Injured Tissue and Capillary or Uninjured Tissue.	100 μ	50 μ	25 μ	10 μ
Possible Potential Differences between Injured and Uninjured Tissues in Volts.	*Drop in Potential in Tissue in Volts per cm.*			
0·001	0·1	0·2	0·4	1·0
0·005	0·5	1·0	2·0	5·0
0·010	1·0	2·0	4·0	10·0
0·050	5·0	10·0	20·0	50·0
0·100	10·0	20·0	40·0	100·0

Fürth, discussing the potential differences which may occur at boundary surfaces in the living organisms, states that if the thickness of a boundary be taken as 10^{-6} cm. the fall of potential between the two surfaces might equal 100,000 volts per cm. theoretically. Such enormous differences are not actually attained because there is not complete insulation.

From Abramson's calculations there appears to be no difficulty in accepting an explanation of exudation and diapedesis based upon the presence of electric fields within the tissues concerned.

(*f*) *The Size of the Transported Particles.*—Although the velocity of their electrophoresis in water is independent of the magnitude of the particles, yet their size seems to have an important influence on their localisation from the bloodstream; for experience has shown that, speaking in general terms, smaller particles become localised from the bloodstream more readily than larger ones—presumably because the hindrance to their passage through the capillary endothelium is less. Thus pigmentation of the skin occurs in response to pressure and other slight stimulations. In laboratory experiments on animals it is found that colloidal dyes—isamine blue and Congo red, for example—when injected into the bloodstream, become more rapidly and more intensely concentrated in an inflamed tissue than do the coarser suspensions of Indian ink or mercuric sulphide in like circumstances. Similarly, viruses appear to be localised by inflammation with more certainty and regularity than are bacteria; and it is noteworthy that the virus of foot-and-mouth disease, which is said to be the smallest virus known (see Galloway and Elford), will, like the natural pigments, become deposited

in tissues which have been "injured" by mere compressions and frictions which are within the bounds of normal physiology. In this connection, too, it may be remembered that arsenical cancer is especially prone to occur in cutaneous regions submitted to what may be described as mild but frequent physiological stimulation, and it is hard to suppose that arsenic is conveyed in the blood in any other than an ultramicroscopic form.

In the present state of knowledge to draw satisfactory comparisons between the sizes of different bacteria and the readiness with which they find an entry into inflamed tissues is not easy. A remarkable fact is the constancy with which the *Treponema pallidum* finds its way into fields of irritation. This may be due to its effective dimensions being reduced by deformation during its passage through the capillary endothelium. At any rate it is known that the agent of syphilis will pass through filters which restrain most bacteria. Whether this capacity to penetrate filters is due to its powers of distortion or to the existence of the treponema in another or ultramicroscopic form, as some (Lépine) have suggested, may for the present be left an open question. Admittedly the effective size of any organised structure cannot be gauged by its appearance when at rest under the microscope. If it were so there would be difficulty in accounting for the early diapedesis of the white blood corpuscles. Direct observation shows that these structures undergo great distortion as they pass through the capillary wall, and the plausible suggestion has been made that the peculiar shape of the nucleus of the polymorphonuclear leucocyte is a special adaptation to facilitate the escape of the cell through the vascular endothelium.

Harrop, Spagnol[3] and others have devoted attention to the permeation of the walls of the fine bloodvessels, and have concluded that the size of the particles plays a leading part in the ease with which they traverse the capillary endothelium.

(*g*) *The Electrical Charge on the Particles.*—The blood being alkaline, the inner surfaces of the bloodvessels, the cells and the colloids of the circulating stream all carry negative electrical charges. Any substance, therefore, which is injected in large amounts into the bloodstream must also bear a negative charge if it is to circulate freely, for positive substances so introduced will become adsorbed at once on to the walls of the bloodvessels and on to the electronegative constituents of the blood. For this reason, perhaps, electropositive substances given intravenously are apt to have injurious effects, and in any case show little or no tendency to become concentrated in the macrophage system.

Von Jancsó[1] found that when arsenious acid in distilled water was given intravenously to rats it caused no damage to the reticulo-endothelial cells, but when adsorbed by Indian ink and so introduced it brought about reticulo-endothelial catastrophe within thirteen hours. He was able also to poison the reticulo-endothelial cells with the electropositive dye Janus green by adsorbing it to Indian ink. Though toxic for the animal, Janus green given alone in watery solution does not become localised in the reticulo-endothelium.

Spagnol[3] states that negatively charged colloidal dyes introduced into the bloodstream always become localised in areas of skin where irritation is caused, while positively charged dyes never become localised in this way

even though the amount injected is enough to colour the whole of the animal's body.

Spagnol found, like von Jancsó, that by adding an electropositive dye to an electronegative dye the charge on the former could be reversed, and it would then be taken up by the phagocytes of the reticulo-endothelial system. For example, 50 c.c. of a 0·5 per cent. solution of alizarin red when added to 50 c.c. of a 0·1 per cent. solution of Janus green, would change the charge on the latter and the combination of the two dyes would then become fixed in the macrophage system.

The writer has made a few observations on the subject which accord with the preceding ones up to a point (p. 76). But it appears to him that disjunction of two colloidal dyes united *in vitro* in this way may perhaps occur gradually after their introduction into the bloodstream.

Discussion.

Viewed in the light of the foregoing arguments, life would appear to consist largely of the maintenance of potential differences at the cell boundaries by a supply of oxygen upon the one hand and by metabolic processes on the other. With cellular injury the conductivity of the intervening phase is diminished and the potential differences fall; with an increase of injury and conductivity the differences of potential will disappear and, unless restored, life will cease. Applying this to the vascular reactions occurring in the complex organism, one may suppose that the inner surface of a capillary bloodvessel carries normally a negative charge, while the tissues immediately outside the capillary, where the oxygen tension is less and metabolites are in higher

concentration, will carry a relatively positive charge. Supposing the conductivity of the endothelium to remain constant, the potential differences between its inner and outer surfaces will remain constant also, for the cytoplasm being to some slight extent a conductor, there will be an automatic regulation of the potentials. An increased metabolism, by raising the potential outside the capillary while that of its inner surface is unchanged, will cause an equivalent increase in the transfer of ions. In such a way there can be envisaged a mechanism for regulating the interchanges between the blood and the tissues in accordance with their requirements from time to time. This may be looked upon as the condition in health.

If, on the other hand, through any cause the " permeability " of the capillary wall is increased, there will be at once a transfer by electrophoresis of electronegative particles from the bloodstream to the tissues outside, with a consequent lowering of the potential differences within and without the capillary wall. In other conditions—an increase of metabolism, for example—there might be a rise of potential outside the vessel resulting in greater exudation from the capillaries without their " permeability " being of necessity increased; and if such a supposition be correct, it is clear that to refer all increased exudation to an increased permeability of the capillary walls would be erroneous.

Many hitherto unexplained though recognised facts of physiology find a ready explanation in the hypothesis just advanced. For example, the response of tissues to pressure and strains (Wolff's law) by hypertrophy may be regarded as due to rises of tissue potentials in

response to irritation and increased function causing an increased supply of the materials required for growth. The refractory or irresponsive state following wheals too can be explained in this way: suppose, owing to an injection of histamine, the walls of the capillary vessels in a certain region are rendered suddenly more "permeable," there will follow a transfer of electronegative blood proteins from these vessels into the less electronegative tissues outside. This inflammatory effusion will be accompanied by a neutralisation of the difference of potentials. Consequently no recurrence of effusion will be caused by a repetition of the stimulus until the vessel walls have recovered their normal resistance to electrical conduction and the proper degree of potential difference has been established between the inner and outer surfaces of the capillary walls.

Again, if we suppose that, owing to repeated injury—as, for example, exposure to cold—the damaged capillary vessels in any region do not regain their normal degree of impermeability, there will be a failure to maintain the normal potential differences on the inner and outer sides of the capillary walls and the amount of exudation in response to any sudden injury will in consequence be diminished.

Yet one more point in favour of the above explanation of inflammation is the fact that electropositive colloids when injected into the tissues bring about an inflammatory response with exudation.

CHAPTER XIII

THE RETENTION OF COLLOIDS AND OTHER SUBSTANCES BY INFLAMED TISSUE

THE main theme of previous chapters has been that inflammation and certain other conditions are accompanied by the passage of plasma, fine inorganic particles, viruses, bacteria, and leucocytes from the small bloodvessels into the perivascular spaces. Having reached this situation it might be supposed that the substances concerned would drain away, more or less speedily, in the lymph stream. In the presence of established inflammation, however, no such relatively quick removal comes about. The retention of transuded lymph is familiar to everyone as inflammatory œdema and serous effusion; and the local retention of the polymorphonuclear leucocytes which have undergone diapedesis is also generally recognised, especially if the pathological disturbance has led to the formation of an abscess. That the principle holds good for other matters than lymph and blood cells has been sufficiently demonstrated already in the earlier pages which are concerned with the diapiresis of dyes and fine-coloured particles; for it will be clear that the colouring matters mentioned in many of the instances recorded in those pages not only escaped from the bloodstream into the inflamed tissues, but were retained there.

It is not to be denied that œdema may follow a rapid production of lymph, even though neither inflammation nor lymphatic obstruction is present. But this kind

of retention is merely due to a temporary excess of input over output, the flow of lymph being normally a sluggish process; moreover, the œdema in such a case, provided that its cause is a local one, will disappear with such a change of posture as to allow gravity to assist the depletion. Whereas œdema brought about by continued inflammation will not subside under the influence of gravity so long as the inflammation continues. An œdema which is transient only, it is true, may be caused by irritation when this is of short duration. Whealing of the skin in response to histamine introduced by pin-pricks is an example. In this case the causal agent (histamine) becomes removed or inactivated, and there is no persistent obstacle therefore against a return to normal conditions. With infected wounds the irritation is more lasting, and therefore the œdema is maintained.

Apparently the conditions which lead to the local retention of lymph and other substances, including bacteria, are not always established directly on the receipt of a wound. This time interval, if it exists, must be a matter worth consideration in connection with the spread of infection from freshly made wounds.

Some of the recorded experiments which show explicitly the holding up of colloids and other bodies by inflamed tissue will be set out here. And to avoid ambiguity it may be well to explain that the following remarks are concerned with a primary and more or less temporary retention due to changing vital processes, and not with the secondary and more durable retentions, an example of which is the adsorption of silver by collagen and reticulum fibres as seen in argyrosis.

Opie,[1, 2, 3, 4, 5] in a series of papers, has discussed the

arrest and concentration of antigens in inflamed tissues. He observed, while immunising rabbits against egg-white or horse serum, that these substances, after repeated injection into the peritoneum or the subcutaneous tissues, entered the bloodstream with decreasing readiness. In well-immunised animals these antigens failed entirely to enter the circulating blood unless given in massive dosage. In a non-immunised animal, however, egg-white and horse serum readily passed into the bloodstream, where they could be detected by precipitin tests for seven, eight, or nine days after their introduction into the subcutaneous tissues. His explanation is that when an antigen meets its antibody in the living organism an acute inflammation is at once produced in the tissues wherein they meet. This inflammation has the double effect of (*a*) preventing a free distribution of the antigen, and (*b*) causing a copious inflow of antibody owing to the increased transudation which results from the inflammation. The Arthus phenomenon, as displayed in the tuberculin reaction and other skin tests, thus represents a protective arrangement whereby an antigen is fixed and destroyed at its portal of entry.

As the work of Opie and of Menkin, who also has followed up this matter of the retention of foreign proteins in inflamed tissues, has been discussed already (p. 46), no further reference will be made to the matter here.

Okuneff[1] observed that the absorption of trypan blue from the peritoneal cavity was delayed if animal charcoal, casein, or gelatine were injected simultaneously with the dye or thirty minutes previously.

In some further experiments Okuneff[2] inquired into the effects of hyperæmia and inflammation upon the

rate of absorption of a dye injected into the subcutaneous tissues. He used rabbits. A 1 per cent. solution of trypan blue was injected subcutaneously in the loin, and at successive intervals afterward the amount of trypan blue in the blood was estimated. This gave the standard rate of absorption for the rabbit used. Eight or more days after the primary test the procedure was repeated under various modifying conditions, the trypan

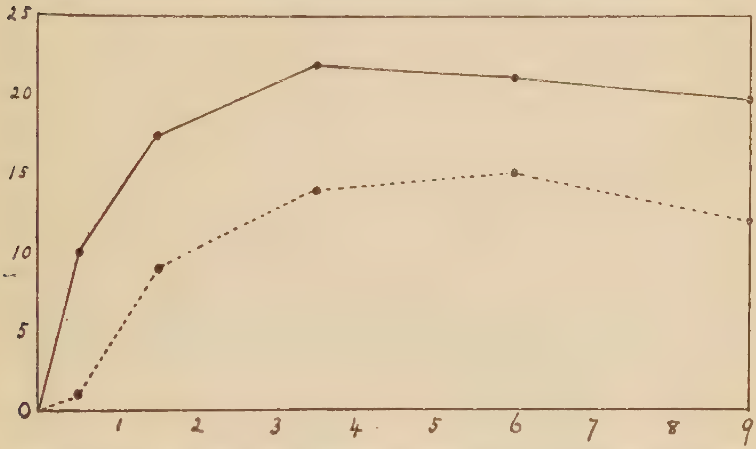


FIG. 2.—In this and the following figures the ordinates represent that percentage of dye injected which is estimated to be present in the blood. The abscissæ denote hours following the injection. The unbroken line represents the normal rate of dye absorption; the dotted line shows the rate of absorption under the influence of locally applied cold.

blue being injected into the intact loin. In this way he found that vaso-constriction caused by a previous injection of adrenalin at the site of introduction of the dye delayed its absorption. A rubber bag filled with ice applied to the loin for forty-five minutes, the dye being injected within five minutes of applying the bag, also resulted in a delayed absorption of the dye (Fig. 2). Heat, applied by a hot-water bottle so as to preserve

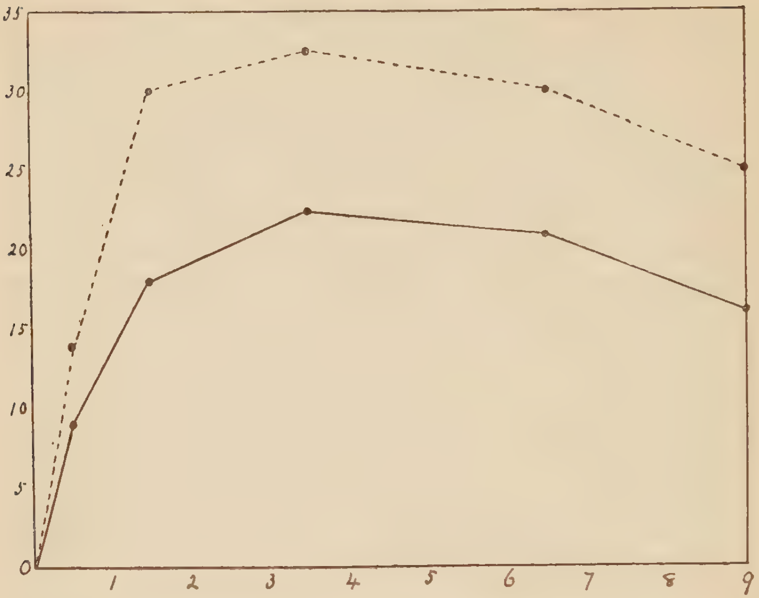


FIG. 3.—The unbroken line shows the normal rate of dye absorption; the dotted line indicates the rate of absorption under the influence of locally applied heat.

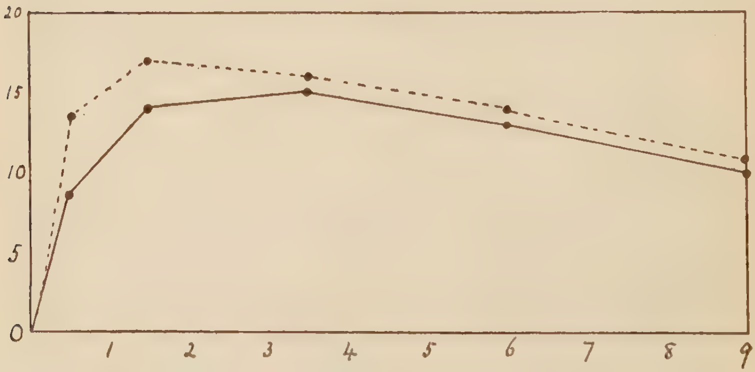


FIG. 4.—The unbroken line represents the normal rate of dye absorption. The dotted line shows rate of absorption after application of a mustard plaster at the site of injection.

a temperature of 35 to 45° C. between the animal's skin and the bag, caused an increased rate of absorption (Fig. 3). The same result followed hyperæmia due to the application of a mustard plaster, the dye being injected within ten to twenty minutes of applying the plaster (Fig. 4). Experiments carried out with turpentine were of especial interest. If a small amount

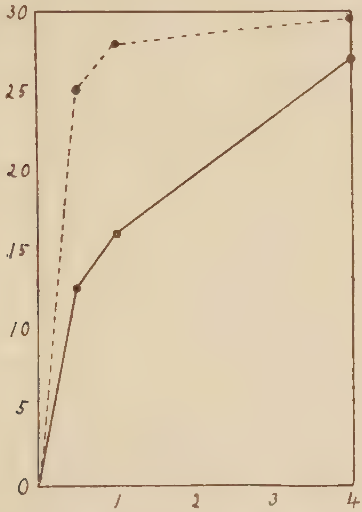


FIG. 5.—The unbroken line represents the normal rate of dye absorption. The dotted line shows the rate of absorption when 0·2 c.c. of turpentine was injected subcutaneously immediately before the dye.

of turpentine (0·06 to 0·2 c.c.) was injected subcutaneously in the loin immediately before giving the dye, an increased rate of absorption occurred (Fig. 5); but if the trypan blue was injected twelve hours after the turpentine, a great reduction occurred in the rate of absorption of the dye (Fig. 6). These differences are well shown in the accompanying charts, which are reproduced by Dr. Okuneff's kind permission. The

results are of considerable importance, bearing as they do upon the time interval requisite for the establishment of conditions favourable to retention.

Kusnetzowsky[2] noticed that trypan blue and lithium carmine, given intravenously, appeared in excessive quantity in parts of the body which were subjected to venous congestion, but that no corresponding storage of these dyes by the macrophages took place in the congested tissues. Whereas when localised under the

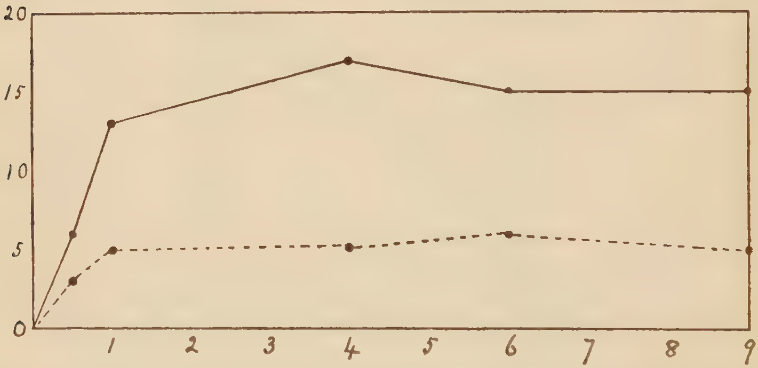


FIG. 6.—The unbroken line represents the normal rate of dye absorption. The dotted line shows the rate of absorption when 0·2 c.c. of turpentine was injected subcutaneously twelve hours before the dye.

influence of hyperæmia induced by heat these dyes were taken up by the tissue macrophages and retained.

It is difficult to be sure when producing hyperæmia by heat that the tissues have not been injured to the extent of producing an inflammatory reaction. The writer's experience confirms the observations of Rogowicz (p. 29) in which, although a dye given intravenously appears in a larger concentration in hyperæmic tissue than elsewhere, it shows little tendency to be retained there unless the stimulus causing the hyperæmia has

brought about inflammation also. The size of the dye particles has to be considered in such experiments.

Menkin,[1] on repeated occasions, injected trypan blue into the foreleg of a normal rabbit and collected the lymph returning from the limb by catheterising a subclavian lymphatic vessel, and he found that the dye readily appeared in the lymph so collected. In other rabbits he caused an aseptic inflammation of the forelegs by injecting a mixture of aleuronat and starch an hour or so before injecting the trypan blue, and in these animals the dye did not appear in the lymph. In another series of experiments he caused aseptic peritonitis by injections of aleuronat, and half an hour later he gave intraperitoneal injections of trypan blue. The dye was retained in the inflamed peritoneum so that the retrosternal glands showed no trypan blue or only a trace of it. In normal control rabbits to which similar doses of trypan blue were given, the dye was more quickly removed from the peritoneum, and the retrosternal lymphatic nodes became deeply stained.

The fact that the capillary endothelium of the frog is normally permeable to colloids, including proteins, has been mentioned previously. In spite of this vascular permeability, the retention of substances in tissues which are inflamed occurs in frogs as it does in mammals. Menkin[4] injected a small amount of diluted croton oil into the subcutaneous lymph space of a frog's thigh. After an interval varying from forty-five minutes to one day, he injected 0·5 c.c. of a 1 per cent. solution of trypan blue into the inflamed portion of the thigh, and made a similar injection into the same part of the thigh of a normal frog. After several hours both animals were pithed and examined.

In the control frog the dye had diffused through the body, staining the buccal cavity, tongue, abdominal wall and thighs, viscera, and heart. The experimental frog, on the other hand, while characterised by intense staining at the site of inflammation, showed in general no dye in the buccal cavity, tongue, or abdominal wall, while the normal thigh, the visceral organs, the heart, and the blood also were as a rule devoid of any trypan blue.

Regarding the rapid dissemination of bacteria in normal animals Noetzel[2] observed that *B. pyocyaneus*, when injected into the knee-joint of a rabbit, could be demonstrated in the inguinal lymphatic glands within ten minutes or less. Buxton, and Wells and Johnstone recorded that bacteria injected into the peritoneal cavities of normal animals reached the bloodstream *via* the lymphatics within a few minutes.

Schmidt-Ott noticed that trypanosomes, spirochætes, and certain bacilli may be found within the regional lymph nodes within a few minutes after their subcutaneous inoculation, and he formed the opinion that the rapidity of this dissemination to the lymph nodes was not dependent on the size, motility, or virulence of the infectious agents, although he thought that as regards the entry of organisms into the general bloodstream virulence played a decisive part.

Pawlowski, however, found that if a suspension of *S. aureus* were injected into the knee-joint of a guinea-pig in which inflammation had been brought about by the introduction of turpentine four days previously, dissemination of the organism was delayed or entirely prevented.

Similarly Sicard, Paraf, and Wallich found that an

aseptic peritonitis produced by an injection of tapioca prevented the dissemination of cholera vibrios and other organisms introduced into the peritoneum.

Opie[5] states that streptococci introduced into the peritoneal cavity of a healthy rabbit entered the blood-stream within a few minutes, whereas the presence of an aseptic peritonitis set up by an injection of aleuronat forty-eight or seventy-two hours beforehand, entirely prevented the micro-organisms from becoming disseminated.

Willis, following up some observations previously made while working with Krause, demonstrated clearly the prevention of dissemination of tubercle bacilli as the result of an immunity response at the site of inoculation. His experiments may be summarised as follows:

(1) Intracutaneous inoculations were made into normal guinea-pigs at a point halfway between the axilla and the groin. Human bacilli of average virulence (H37) were used, 0·1 c.c. of a thin emulsion being injected. The point of injection was marked by a ring of carbol fuchsin. One hour later, or at longer intervals, the inoculated skin was lifted up and removed with scissors after careful surface disinfection with 70 per cent. alcohol. The subsequent occurrence of tuberculous lesions in the lymph nodes or viscera showed that the spread of bacteria must have taken place speedily after the inoculation in these animals, for secondary lesions sometimes came about in animals from which the focus had been excised within one hour of inoculation, and secondary lesions always developed in animals from which the focus of inoculation had been removed after an interval of three hours or more.

(2) Twenty-four normal guinea-pigs were inoculated with the same material in the same site and with the same dose as those in the former experiment. At varying intervals afterward the axillary and inguinal lymph nodes were excised and ground with sand in a mortar. Normal animals were then inoculated with this material. Most of these test animals, given material removed twenty-four hours or more after the injection of the bacilli, developed lesions, thus showing that the introduced bacilli had spread from the point of introduction to the lymphatic glands, about 5 cm. away, within twenty-four hours.

(3) Experiments similar to the foregoing were carried out on forty guinea-pigs which had been inoculated subcutaneously in the right groin with 0·5 c.c. of a heavy suspension of tubercle bacilli seven weeks previously. For this primary inoculation a strain (R1) was used which "is a tubercle bacillus known to initiate a lesion but not progressive disease after subcutaneous inoculation of the dosage stated." It was found in these animals that excision of the secondarily inoculated tissue as long as six days after the injection prevented dissemination of the infection. Transit of the tubercle bacillus in these animals from the site of the second inoculation to the nearest lymph nodes always took more than two weeks.

Operations on the human subject afford support to some of these experimental findings. The suggestion was made at one time that in cases of peritonitis benefit might accrue from drainage of the thoracic duct; this structure, however, proved difficult to find owing to the fact that in the presence of peritonitis it was nearly or quite empty.

A few direct observations have been made regarding the retention by inflamed tissues of inorganic substances. Menkin[2] gave intraperitoneal injections of 5 c.c. of dialysed iron (" colloidal ferric hydroxide with 5 per cent. ferric oxide and ferric chloride "), and subsequently examined the retrosternal lymphatics by the Prussian blue reaction. He found that whereas in normal rabbits iron could be demonstrated by this means in the retrosternal lymph nodes, in rabbits with peritonitis no iron reached these lymphatics.

A striking example of the retention of fine inorganic particles in tissues which are the seat of chronic inflammation is anthracosis. For, as Cummins and Sladden have shown, no remarkable degree of anthracosis occurs in coal-miners unless silica also has been inhaled. In the absence of inflammation set up by the presence of silica the relatively non-irritating coal-dust is not retained in the lung in amounts comparable to those which are so retained in the presence of silicosis. Reference will be made later to the association of pulmonary tuberculosis with silicosis (p. 277).

Much further evidence of the retention of various substances, including micro-organisms, in inflamed tissues is to be found in the copious literature concerning fixation abscess. The fact itself, in one form or another, must be known to every clinician, and there is no need to labour for additional proofs.

Discussion.

A natural outcome of the foregoing facts might be the question whether the escape of colloids and other substances from the blood into the surrounding tissues

is always accompanied by their retention there, as happens in the presence of inflammation. In other words, are diapiresis and local œdema invariably associated? Further experimental work must be carried out before a satisfactory answer can be made.

That increased permeability of the capillary endothelium may sometimes be associated with an increased rather than a delayed rate of lymph removal is at first sight, perhaps, suggested by certain clinical observations made by means of the intradermal salt test for latent œdema, introduced by McClure and Aldrich. This test is performed by injecting into the skin 0·2 c.c. of a 0·8 per cent. aqueous solution of sodium chloride, and noting the time taken by the resulting artificial wheal to become impalpable. McClure and Aldrich discovered that this disappearance time varied inversely with the amount of general œdema present. In healthy individuals an hour or more was required, but in patients with œdema this interval was much reduced; and in nephritis, even if no pitting on pressure could be detected, the time required for complete disappearance of the artificial wheal was diminished. Cohen found this test to be of value in determining the adequacy of the arterial circulation. Thus, two hours after ligation of the femoral artery and vein in a rabbit, artificial wheals were produced in both hind limbs; the wheal in the limb with the obstructed vessels persisted for thirty-five minutes only as compared with sixty minutes for the normal side. Even more striking were the findings of Cohen, Applebaum, and Hainsworth in cases of obliterative endarteritis, for in patients with this condition not only was the disappearance time of artificial wheals in the affected

limb greatly reduced, but it gave a more or less reliable index as to the approach of gangrene. It is known that arterial or venous obstruction leads to increased permeability of the capillaries within the area of distribution of the occluded vessel, so that at the first glance it might seem that here we have an example of increased permeability of the capillaries unaccompanied by increased retention in the tissues. But we cannot build too much on an analogy between an " artificial wheal " of salt and water and a naturally occurring local œdema. The rapid disappearance of an " artificial wheal " in situations where the blood supply is deficient may be attributed, perhaps, to other causes—for example, to an increased avidity of the tissues for water owing to a local relative acidity consequent upon the deficient circulation of blood, or it may be attributed to the increased permeability of the capillary endothelium itself. Starling and Tubby produced experimental evidence which suggested that indigo carmine and methylene blue injected into the tissues or into the serous cavities became removed therefrom by the bloodstream rather than by the lymphatics.

The Causes of Inflammatory Retention.

(*a*) *An increased osmotic pressure* in the transuded material may be one of these. That inflammatory exudate has a larger protein content than normal lymph is well known. Ritter was led to believe that the raised tension in an abscess was due to hypertonicity of the inflammatory exudate by the fact that pus serum was shown by cryoscopy to have a higher osmotic

pressure than blood serum. Digby, Pollard, and Catto arrived at a similar conclusion in consequence of some experiments which they carried out in order to determine the electrical conductivity of inflammatory exudates. The pus of abscesses in the connective tissues they found to be consistently hypertonic. Septic effusions into serous cavities did not show the same consistency—some being hypotonic. They suggest that the hypertonicity is brought about by the katabolic action of proteolytic ferments in the pus and bacteria of the abscesses. Ebbecke explains the fact that a wheal persists while the capillaries from which it has been derived remain patent as due to the large osmotic pressure of the exudate hindering reabsorption which normally depends upon the difference of osmotic pressure between plasma and the tissue liquid.

(*b*) *Stasis of Lymph.*—The writer is not aware of any direct observations which have been made upon the flow of lymph through the finer lymph channels in irritated tissues, and it is only possible therefore to consider the subject upon hypothetical grounds. Stasis and its resolution, as seen in the small bloodvessels, are familiar consequences of irritation (p. 13). The suggestion arises that a similar phenomenon may take place under the like conditions in the small lymphatics; and seeing that the circulation of the lymph is much slower than that of the blood, it might be supposed that stasis would be more easily caused in the lymphatics. Even if proved to occur, lymph stasis must remain nothing more than a partial explanation until it is known what are the underlying forces which bring it about. At present the suggestion that a lymph stasis

akin to capillary blood stasis may happen lacks a foundation of ascertained fact.*

(*c*) *Clotting of Lymph.*—That lymph which exudes into the connective tissue spaces in consequence of inflammation forms clot was noticed very many years ago, and led to a recognition of the fact that inflammatory exudate consisted of blood plasma.

Opie found that dogs, after intramuscular injections of cantharidin, suffered from nephritis and œdema of the liver and gall-bladder. The lymph nodes of the liver were enlarged, and microscopical examination showed an immense dilatation of their lymph sinuses. Within such sinuses a fibrinous coagulum formed a network with fine meshes. Opie was of opinion that this clotting of lymph in the sinuses of the lymph nodes was sufficient to obstruct the natural flow.

Menkin[4] has recently made a careful inquiry into the matter. He brought about cutaneous inflammation in a rabbit by introducing a culture of *Staphylococcus aureus*. After a sufficient time he injected 1 c.c. of a 1 per cent. solution of trypan blue into four or more areas of the skin adjacent to the site of inflammation. The inflamed area became circumscribed by a band of blue, the dye failing completely to penetrate the œdematous tissue. He performed similar experiments on frogs, causing local inflammation by means of diluted croton oil, and subsequently injecting a solution of trypan blue into adjacent areas. No dye diffused into the inflamed part. Often the dye would be seen bulging against the fibrinous septum at the periphery

* A paper by McMaster and Hudack, which I had not seen when the above was written, states that the lymphatics of regions that are inflamed may become so permeable as to fail largely, if not entirely, in the function of drainage.—H. B.

of the zone of inflammation without actually penetrating into it. Menkin explains this failure of penetration by the occlusion of lymphatic vessels and the presence of a network of fibrin in the inflamed area; and he regards the fixation of foreign substances by the inflammatory reaction as due to mechanical obstruction caused by a network of fibrin and by thrombosed lymphatics at the site of inflammation.

The author, after reading Menkin's paper, carried out the following experiment. A patch of skin on the back of a white rabbit was epilated by means of sodium sulphide. A fortnight later a wheal was produced in the epilated area by applying to the skin a rectangular strip of filter-paper moistened with chloroform. One hour later two intradermal injections of diluted Indian ink were made close to the edges of the wheal, the needle being parallel with the wheal edge while the injection was being made. The ink, while diffusing into the normal tissues, did not invade the area of the wheal (Plate VI., B).

In another paper Menkin records that (i.) *B. prodigiosus* injected into inflamed tissue is fixed there and fails to disseminate to the regional lymph nodes as readily as when injected into normal tissue; and (ii.) *B. prodigiosus* inoculated at the periphery of an inflamed area does not readily penetrate into the site of inflammation. The experiments furnish additional evidence, he believes, that the fixation of foreign substances by the inflammatory reaction is primarily due to mechanical obstruction caused by a network of fibrin and by thrombosed lymphatics at the site of inflammation.

(*d*) *Phagocytosis.*—The part played by the polymorphonuclear leucocytes and the macrophages of

Localisation of dye in skin injured by pressure.

A. A Bier's [illegible] applied to an epilated area of skin in a rabbit for $6\frac{1}{2}$ minutes with a [illegible]ative pressure of 6 cm Hg. Six minutes later isamine [illegible] given intravenously. The dye has become localised only where the [illegible] been pressed upon by the rim of the cup.

Resistance of inflamed tissue to the diffusion of Indian Ink.

B. A rectangular wheal (X) has been made by applying chloroform to the skin of a rabbit. After an interval, two intradermal injections of Indian ink have been made close to the edge of the wheal. The ink has not passed

inflamed tissue in preventing the spread of infection will be discussed in connection with the nature of inflammatory barriers. Meanwhile it seems safe to assert that phagocytosis plays an appreciable part in the retention of bacteria, dyes, and other foreign substances in tissues which are inflamed. Falk and Matsuda have observed that phagocytosis of bacteria is largely influenced by the electric charge which they carry, and that it is possible to effect alterations of this charge by artificial means; and Falk advanced the view that the virulence of micro-organisms is dependent upon their electric charge.

(*e*) *Agglutination of Bacteria in vivo.*—Metchnikoff, discussing phagocytosis, referred to substances sometimes present in the blood plasma, which acted upon invading micro-organisms by rendering them motionless and agglomerating them into masses. Tsuda examined the response of the subcutaneous tissues of normal and immunised mice respectively to local injections of streptococci and pneumococci. He found that, whereas in non-immunised animals virulent bacteria underwent but little phagocytosis and were rapidly disseminated, when injected into immunised animals the same bacteria became agglutinated, underwent rapid phagocytosis, and failed to disseminate. Cannon and Pacheco carried out a somewhat similar inquiry, using a virulent culture of *Staphylococcus aureus* as the infecting agent, and guinea-pigs as the reactors. For immunisation purposes a twenty-four-hour growth on agar slants was suspended in 1 c.c. of a 0·9 per cent. solution of sodium chloride and heated to 60° C. for one hour. Of this vaccine 0·2 c.c. was injected into the skin of the abdominal wall on each of ten successive

days. Twenty-five days later tests were made by introducing virulent cultures into the prepared area, and comparing the results with those produced in animals which had not been immunised. Six hours after the injection of a virulent culture of *S. aureus* into normal animals numerous leucocytes were present and were phagocytosing the staphylococci, but there was no evidence of localisation of the infection or of injury to the staphylococci. Six hours after a similar injection had been made into an immunised animal there was found to be an enormous infiltration of the tissue with inflammatory cells containing a rich proportion of lymphocytes and monocytes, while there was no evidence of spreading of the bacteria which had undergone clumping and were no longer present as separate individuals.

With regard to the fixation of a foreign protein under the influence of an immunity response it may be, as Opie[4] has suggested, that specific precipitation plays a part (p. 47).

(*f*) *Electrostatic Conditions in the Tissues.*—Although the suggestion must remain vague, seeing that precise data are lacking, it appears reasonable to suppose that the retention of foreign substances in tissues which are inflamed may be due, in part at least, to electrostatic conditions. There are grounds for believing that an inflamed tissue is charged in a positive sense relatively to normal tissues, these indeed being positive relatively to the blood. With such relationships, the effusion of plasma into the extravascular tissues which follows any circumstance causing an increased permeability of the capillaries can be understood. Inflammation, being accompanied by an increased permeability

of the bloodvessels and by an augmented difference of electrical potential between the contents of the bloodvessels and the extravascular tissues, would not only result in a transference of the mobile electronegative colloids of the blood to the positively charged tissues, but until neutralisation of the charges had taken place to some extent, the effused substances would be held within the damaged zone by electrical attraction.

(*g*) *The electrical charges on the particles which are retained* under the influence of inflammation must be of importance if the foregoing theoretical argument is sound. On such a basis it is as easy to account for the rapid spread of negatively charged bacteria through non-inflamed electronegative tissues, as it is to understand their retention in inflamed tissue under the influence of the raised electrical potential. The same argument can be applied to the retention of proteins, acid dyes, and other electronegative substances under the influence of inflammation. In continuation of this line of thought reference may be made to the concentration of anions in tissues which are inflamed. It has long been known, for example, that chlorides become concentrated in a pneumonic lung. L. Loeb has explained the phenomenon in this way: "There is reason for assuming," he says, "that in pneumonia, as in all foci of inflammation, the concentration of the hydrogen ions is increased, and it has been suggested that possibly as a result of this change the proteins in the exudate become converted into kations and therefore begin to bind the chlorine ions."

The iso-electric point of fibrin is said to be in the neighbourhood of pH 7·2 (Kugelmaas), which is well within the limits of physiological variations of hydrogen-

ion concentration in living tissues. When it passes from the alkaline to the acid side of its iso-electric point fibrin will become changed—confining our attention to its relationship with NaCl—from a sodium proteinate to a protein chloride. And we may be able to apply this explanation to such instances of the fixation of anions by inflamed tissue as have been already reported. Indeed, the principle is one which, perhaps, can be applied generally. Fundamentally it is comparable with that which leads to the retention of electronegative colloids.

In connection with these remarks it is to be noted that, unlike fibrinogen, most of the plasma proteins are isoelectric at a point which is further toward the acid side than any caused by physiological changes or minor pathological disturbances. Consequently these proteins will be retained in a tissue rendered acid through inflammation or other cause.

PART III

GENERAL DISCUSSION

CHAPTER XIV

THE INFLAMMATORY BARRIER

Every surgeon is aware of the potency of an inflammatory barrier in limiting the spread of micro-organisms, and habitually makes use of this knowledge in the treatment of local infections whether situated within the abdomen or in the connective tissues of the hand, or elsewhere. When an abscess is being opened, so long as operative interference is confined within the limits of the surrounding inflammatory barrier, a way will not be opened up for bacterial spread; whereas the incision of uninflamed tissue and the placing of it in sudden communication with a focus of infection will be followed almost certainly by an extension of the disease. Apart from surgical operations, the routine treatment of a local infection consists almost entirely of attempts to second Nature's inflammatory response.

As long ago as 1865 Billroth found that if infected dressings were applied to the backs of dogs where granulating wounds were present, infection of these wounds did not follow. When, however, to prevent their displacement the dressings were stitched into place, although the granulations remained healthy, the stitch holes which had been made in normal uninflamed skin became infected. Afanassieff found that intact granulations formed a barrier against infection with anthrax when a culture of this organism was applied to their surface, and Noetzel made a similar

observation, using cultures of anthrax and of tetanus, although he remarked that any trauma of the granulations sufficient to cause bleeding was enough to break down the defence.

Durham was able to show that the resistance of the peritoneum against infection could be greatly increased by inducing beforehand an aseptic peritonitis. He also noticed that the primary influx of polymorphonuclear leucocytes was succeeded by the appearance of " plasma cells " (macrophages) which were exceedingly phagocytic, and took up the polymorphonuclear leucocytes and debris in addition to bacteria.

Issaeff found that some local and transient resistance against subcutaneous inoculations with cholera could be created in guinea-pigs by previous injections, at the sites of inoculation, of normal human blood serum, bouillon, and even salt solutions. The resistance to infection of the peritoneum also could be raised by similar measures. The slight degree of immunity produced he attributed to increased phagocytosis; and he observed that the preliminary treatment did not give any protection against cholera toxin.

Besredka[1] learned that animals could be protected against a local dermic inoculation of anthrax by previously injecting into that part of the skin some filtrate from a culture of the organism or by the application of the filtrate to the skin by means of a compress. He found later[2] that by comparable means a local protection could be produced against a staphylococcus which was virulent for guinea-pigs. He further observed[2] that a filtrate made from an eighteen-day virulent culture of this staphylococcus was devoid of toxicity when injected into an animal. Staphylococci

inoculated into the filtrate maintained their vitality but did not multiply. When the living organisms were injected subcutaneously together with some of this culture filtrate the resulting lesions were not so severe as those produced by the staphylococci when injected without the filtrate. Similar results (Besredka[3]) were obtained with streptococci and their filtrates. Besredka and Urbain obtained a local protection of guinea-pigs against infection with a virulent streptococcus by previously dressing the skin with bacterial filtrate. Besredka believed that the local immunity was brought about by the presence of specific inhibitory substances in the bacterial filtrates which were used; and in accordance with this view he made application of the method in the treatment of puerperal sepsis and some other infective conditions of man. Brocq-Rousseu, Forgeot, and Urbain confirmed the possibility of obtaining local immunity in guinea-pigs against intradermal injections of a virulent streptococcus by previous inoculation with a culture which had been attenuated by heating it to 60° C. for fifteen minutes.

Citron and Picard used polyvalent filtrates for the treatment of erysipelas, furunculosis, carbuncles, and osteomyelitis. They seem to have taken for granted that the action was specific.

In view of more recent work it may be doubted if the effect of these bacterial filtrates is to any great extent the consequence of specific antibactericidal properties; for other experimenters have created a local non-specific resistance to infections by a variety of means, all of which possess the common property of setting up an aseptic inflammation in the tissues to which they are applied. Thus Arloing and Langeron obtained

local protection for the skin of guinea-pigs against *B. pyocyaneus* by previous injections of plain bouillon into the same area. Rivalier, using rabbits, inoculated (*a*) freshly epilated skin, and (*b*) skin which had been epilated five days before, with a virulent streptococcus. In group (*a*) there followed acute infection, and in (*b*) no infection became established. Two zones of skin treated in the same way in one rabbit gave the same result. Epilation in these experiments was performed by plucking out the hair. By means of plain broth Gratia obtained a similar non-specific protection of the skin in guinea-pigs against staphylococci and also against anthrax. He found, too, that the previous application of vesicants made the skin more resistant to infection, the protection lasting about a fortnight. Mallory and Marble produced a local cutaneous immunity in rabbits toward a staphylococcus equally readily by means of sterile filtrates of cultures in broth and sterile broth alone. The immunity was strictly local, was manifest within eight hours, and lasted for fifteen days at least. The protection obtained by a bacterial filtrate was never greater than that obtained by the original broth from which the filtrate had been prepared. Miller found that guinea-pigs were protected locally against staphylococcal, and rabbits against streptococcal, infection by previous treatment of the skin with horse meat broth or beef tea, or with bacteria-free filtrates of cultures. He regarded the immunity as non-specific. Protection appeared in twenty-four hours, but had disappeared at the end of ten days. He noted that if the inflammation caused by the preliminary treatment were too severe no increase of resistance was obtained. Freedlander and Toomey

also found plain broth as effective as specific broth in protecting guinea-pigs against subcutaneous injections of *Staphylococcus aureus*.

Other pathologists, while admitting the potency of non-specific protection, have obtained a still higher degree of local immunity by the use of specific serum or broth. Gay and Morrison directed attention to the leading part played by the macrophages assembled as the result of aseptic inflammation. They noted the results of injecting streptococci into the pleural cavity of rabbits after the onset of inflammation caused by various substances, and they found that meat-infusion, broth, and diluted egg-white, which at the end of twenty-four hours had produced a notable increase of macrophages in the cavity, protected the animals against one hundred times the minimal dose that was fatal for untreated rabbits. Substances which, when injected into the pleura, brought about a large increase in the number of polymorphonuclear leucocytes but not a pronounced increase in the number of macrophages, were not effective in affording protection to the host. Thus an injection of 5 per cent. aleuronat in 0·5 per cent. sodium chloride gave these "absolute counts" (in millions): macrophages, 3·8, polymorphonuclear leucocytes 58·9; and this injection gave no recognisable protection. Whereas strong broth injected caused an exudate the constituents of which were: macrophages 18·7 and polymorphonuclears 47·9; this treatment caused complete protection. As the outcome of a series of such experiments they regard the immunity afforded by the broth injections as due in the main to the resulting concentration of macrophages. Nakahara came to a similar conclusion. He was able

to enhance greatly the resistance of the peritoneum in mice against certain organisms by injecting into the abdominal cavity forty-eight hours beforehand 0·2 c.c. of commercial olive oil. At first this injection causes an exudation of polymorphonuclear leucocytes, but at the end of forty-eight hours these have become replaced by macrophages. The protection afforded in this way gradually subsides during the ensuing fortnight. The organisms used were *B. coli*, *S. aureus*, and a pneumococcus.

Gay and Clark, and Gay, Clark and Linton found that normal rabbit serum injected into a rabbit's pleura produced just as high a macrophage count as did an antistreptococcal rabbit serum, but the latter led to an earlier sterilisation of the pleural cavity after its infection with streptococci. Macrophages in the animals prepared with immune serum reached their maximum earlier and caused an earlier sterilisation of the pleural cavity than was the case after normal rabbit serum had been used. Rivers and Tillett[2] also observed that immune serum was more efficient than normal serum or beef broth in protecting the skin of rabbits against streptococcal infection.

The observation made by Tsuda and sustained by Cannon and Pacheco, to the effect that agglutination of bacteria occurs in tissues which have been specifically protected, may be recalled in the present discussion. Metchnikoff had already recorded the occurrence of this agglutination of bacteria *in vivo*.

From the foregoing remarks it will appear that to provide the most efficient prophylactic barrier against infection is not quite a simple problem. The matter, considered theoretically, stands as follows: to produce

the most protection it is not enough merely to cause a local inflammation. (*a*) The right kind of inflammation is required—that is to say, a maximum number of macrophages must be mobilised in the zone of infection. (*b*) The level of local immunity must be raised by specific treatment with the object of enabling the tissues to bring about agglutination of the invading organisms. (*c*) The degree of inflammation must be appropriate for the end to be achieved. That this is so might be inferred from the facts already stated earlier in this work in connection with the respective potencies of varying degrees of inflammation in causing localisation of pigments and other materials from the blood. There is, however, some direct evidence on this last matter. Hanger,[2] who was examining the susceptibility of rabbits to infection with *B. lepisepticum*, made the following observations: (1) Animals showing a strong natural reaction towards filtrates of *B. lepisepticum* survive infection by this organism more frequently than weak reactors. (2) Bacterial filtrates injected into the skin twenty-four hours before infection exert a non-specific protection of that area against the organism, even in susceptible animals. (3) Severe injury of the tissues by chemicals or an excessive antigen antibody reaction produces a loss of local resistance even in immune animals. Mild injuries from the same causes, however, have a protective effect. Miller (p. 234) also noticed that if the severity of inflammation was too great no local defence against infection was obtained.

Another fact that prevents the indiscriminate use of inflammation as a panacea against infection or its spread is the necessity of considering the agent against

which protection is required. For some organisms appear to grow as well or even better in an inflamed tissue, or in one that is merely hyperæmic, than in a normal one. Outstanding examples are the agents of syphilis and tubercle, and certain viruses. Most of the evidence is clinical, but it stands upon a base firm enough to be acceptable. As regards syphilis, a great deal that has been said already (p. 91, *et seq.*) upon the localisation of the *Treponema pallidum* in areas of inflammation is applicable to its development in those areas; and experimental work carried out by Chesney and Kemp unreservedly supports these observations. Halley, Chesney, and Dresel showed that while fresh wounds in rabbits were susceptible to infection with *Streptococcus erysipelatis* and *Staphylococcus aureus*, granulating wounds were resistant to these organisms. But this resistance of the granulating wounds was not general against every infection; for they found that *Pasteurella avicida* applied to the surface of the granulations yet gained access to the blood and killed the animals.

With tuberculosis we have not so many clear facts for our guidance, and must rely mainly upon clinical experience; and although this is to be regarded with greater caution than controlled experimentation, it is convincing enough on account of its bulk (pp. 102, 259).

Whether a culture of tubercle bacilli would become established or not when applied to the surface of healthy granulations in a susceptible animal may remain in doubt for the present. But it cannot be questioned that inflamed tissue forms a nidus which is especially favourable to the growth of these organisms.

A mass of evidence is available to show the malign

PLATE VII

Localisation of dye by mild cutaneous irritation.

Chloroform was applied to the right ear of a rabbit, and water blue then injected into the marginal vein of the left ear. The coloured illustration made 24 hours later shows localisation of the dye in the ear irritated by chloroform.

[*To face page* 238

influence of silicosis upon pulmonary tuberculosis; and some experimental work bearing on the subject has been published by Gye and Kettle. They introduced (1) silica alone, (2) tubercle bacilli alone, and (3) silica and tubercle bacilli into the subcutaneous tissues of mice, and they found that the most vigorous lesions were caused when silica and bacilli were injected together. Kettle further observed that although the inflammation set up by local injections of calcium chloride or turpentine was more severe than that produced by silica, yet it was not so favourable to the growth of the tubercle bacillus—an observation which suggests that the grade, or possibly the kind of inflammation, must be considered.

The effects of secondary infections in accelerating the progress of so-called surgical tuberculosis are generally recognised by clinicians, and afford an additional example of the fact that although inflammation increases the local resistance to most, it does not increase such resistance to all bacterial infections.

That viruses become localised in tissues which are inflamed has been amply proved. Whether they prosper there or not remains to be learned, and the elucidation of this point is much to be desired. The virus of measles, if the rash be regarded as an index, will become abundantly localised in skin which has been irritated by an application of mustard. Does the virus find this mild inflammation inimical or otherwise to its prosperity? Similar questions are applicable to smallpox and other virus diseases. It is manifest that if at the same time a virus can be both localised and destroyed a long stride forward in the cure of disease will have been made. If, on the other hand,

it is found that the viruses under consideration multiply most rapidly or are most potent when collected into inflamed tissues, we shall have in this knowledge alone the means toward a more effective control of the diseases concerned. The observations of Jenner upon this matter may be recalled (p. 113).

Unfortunately, there is very little experimental work to give us guidance, and until more is forthcoming it will not be possible to use satisfactorily the knowledge already gained as to the localisation of viruses. The only reference the author has found to an experimental test is a paper by Teague and Goodpasture, who inoculated the previously tarred skin of guinea-pigs and rabbits with material from human herpetic vesicles, and thus produced a zonal herpes with lesions of the corresponding root ganglia; whereas in skin which had not been tarred the "takes" were less severe and the resulting lesions remained strictly local (p. 133).

It is of interest to know that a barrier of granulations will act as a buffer against the absorption of a toxin. Clinical experience suggests that this is true, but there is some direct experimental evidence available. White carried out the following experiment on rats: Under ether anæsthesia an incised wound $\frac{1}{2}$ inch long was made in the gluteal region and a small cavity was made in the muscle into which a plug of cotton-wool was inserted. The skin incision was then sealed with collodion. At varying intervals afterward the wound was reopened, the plug removed, and about twenty times the minimum lethal dose of dry tetanus toxin inserted into the granulating cavity, and the wound was again sealed with collodion. When the period

between inflicting the primary wound and inserting the toxin was more than five days the animals were found to be quite protected from the toxin. Some protection was apparent with shorter intervals, but it was incomplete. This result may be compared with those of Noetzel,[1] who found that in rabbits and guinea-pigs the time required for a wound surface to become resistant to bacterial infection was from five to six days.

In view of the important part played by macrophages in the inflammatory barrier, a word or two may be said as to how they arrive at the places where their activities are required. That they do not all arise from white corpuscles which have migrated from the bloodstream under the influence of inflammation was shown more than sixty years ago by Hoffmann and von Recklinghausen in a paper to which a reference already has been made (p. 55). They excised the cornea of a cat or a frog, cauterised it in the centre, and placed it in the blood, serum, or aqueous humour of the same animal. The preparation was then stored for one, two, or three days; in some experiments in a warm incubator, in others at room temperature. In several experiments carried out in this way they observed a large accumulation of mobile cells around the cauterised spot. These cells were much too numerous in the injured part of the cornea—in one case spreading throughout the whole tissue—for them to represent simple groupings of the corpuscles originally present. They certainly could not have entered from outside. Hoffmann and von Recklinghausen concluded therefore that a genuine formation of new cells had occurred in the corneas in consequence of the burn. At the

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present date these cells can be identified with macrophages in view of the fact that similar cell accumulations which resulted from cauterisation of the cornea in the living animal phagocytosed granules of cinnabar.

In later years Maximow[1, 2, 3] showed by his studies on aseptic inflammation and on tissue cultures that the macrophages have a double origin, and he believed that the relative numbers arising from one and the other of these sources varied greatly according to the nature of the tissue and the stimulant. Some of the macrophages, he observed, arose from the fixed histiocytes which, under the influence of inflammation contracted, rounded off, and became active as large, amœboid, phagocytic, dye-storing cells. The others, and usually the larger number of the macrophages, were derived from the non-granular leucocytes—both lymphocytes and monocytes—which had migrated into the tissue from the bloodstream. After three days, in a vitally stained animal, these blood-derived lymphocytes and monocytes could not be distinguished from the locally derived histiocytes. Mitoses occurred in these hypertrophied lymphocytes and monocytes, but were rare. He also observed that after a week or more some of the resulting macrophages became irreversibly changed into fibroblasts.

The origin of macrophages from the lymphocytes and monocytes of the blood has been observed by many cytologists and appears to be beyond doubt (*cf.* M. R. Lewis, Carrel and Ebeling, Higgins and Palmer, Bloom).

Matters that are of much practical importance in connection with the treatment of wounds arise out of the foregoing discussions. To prevent general dissemination of any bacteria that have entered the tissues

will be the first consideration in treating a freshly inflicted wound. Such prophylaxis one may suppose to be impossible in every case, for micro-organisms may have been introduced directly into the blood-stream when the breach of tissue occurred. By repeated experiments the author found that when a dye was injected into the subcutaneous tissues of a rat, even though a fine (No. 20) hypodermic needle was used, some of the dye was frequently found to enter directly into the bloodvessels. As concerns the practical side of the question, it is to be noted that with open wounds the protecting barrier of granulations requires, in certain of the lower animals, a period of several days for its development, and even then a slight injury, just enough to cause bleeding, may lay open the defences to bacterial invasion. These two facts both give us guidance in the treatment of human patients.

The production of a macrophage barrier in healthy subcutaneous tissue or in the peritoneum or pleura would appear to need twenty-four hours at the least, while it will be effective for perhaps a week or two weeks after its establishment.

With regard to freshly inflicted wounds, we have to pay serious attention to Okuneff's experiments with the absorption of dyes which had been injected into the subcutaneous tissues. He found that hyperæmia accelerated absorption, and that a definite lapse of time was required under the conditions of his experiments to institute those conditions which cause retention of substances under the influence of inflammation.

CHAPTER XV

PERSISTENT ENDOTHELIAL PERMEABILITY

IN earlier chapters of this book attention has been directed to the existence in some individuals of areas of skin where the small bloodvessels remain abnormally permeable during long periods of time. The surface in these regions often is characterised by a dusky redness which may turn to cyanosis in cold weather, and in either case is in contrast with the pinky pallor of the normal skin. The bloodvessels in these affected areas react imperfectly or not at all to applications of adrenalin, pituitrin, or histamine, and they remain for relatively long periods, and maybe always, unduly permeable to substances carried in the blood.

Examples of this condition in the dermis are afforded by (i.) some congenital nævi, (ii.) scar tissue, (iii.) the darker areas of a mottled skin—*erythema ab igne* affording a particularly good illustration—and (iv.) the redder parts of the cheeks and other portions of the body which have been much exposed to excessive heat or cold.

Mottling of the skin is of particular interest. Lewis has shown that the paler areas may be warmer than the dusky ones. Each pale spot represents the district immediately surrounding a small artery and supplied by it. The dusky areas, being more remote from the arterial influx, are less well oxygenated. When it is considered that these darker and less completely oxygenated areas are those in which the bloodvessels

are abnormally permeable, the work of Rous and his colleagues on the gradient of vascular permeability is at once brought to mind, for it seems to provide at least a partial explanation of the excessive permeability of the vessels in the dusky areas as compared with those in the paler spots (p. 40). Rous, Gilding, and Smith found that the permeability of the capillaries gradually increased from the arterial to the venous end; and the darker areas of a reticulated skin represent the venous portions of the small vessels supplying this tissue.

Evidence of the increased permeability of the blood-vessels in these abnormal areas is to be found in the fact that various matters escape from the bloodstream in the affected tissues and become deposited in them. Unna noticed the tendency for pigment to become laid down in the dusky portions of a reticulated skin, a familiar example of which is seen in the legs of people who sit much in front of an open fire. Kelynack and Kirkby, Borsellini, Lehner and Kenedy[2] have commented on the tendency of arsenical pigmentation to take place in the darker reticulations of a mottled skin, and Brooke and Roberts have mentioned its frequent appearance in old scars.

Many writers have remarked on the especial incidence of syphilitic lesions in the darker areas of a reticulated skin, among them being Neumann, Ehrmann, Adamson,[2] Lehner and Kenedy,[1] and Williams and Goodman. Others have described a similar localisation of tuberculosis. Hutchinson[1] noticed that lupus frequently was associated with chilblains and other abnormal conditions of the skin caused by exposure to cold and heat. His observations have been supported by many dermatologists. The tendency for tuberculides to

break out in the darker areas of a reticulated skin has been commented upon repeatedly. Concerning viruses, v. Pirquet mentioned the especial abundance with which a measles rash was apt to appear in or immediately adjacent to scars, and Adamson[1] and Lewis and Harmer found a similar liability for the rash to crop up in the darker areas of a reticulated skin.

In view of such accumulated evidence it seems certain that the bloodvessels in these abnormal areas are unduly permeable and remain so for relatively long periods.

Among the possible causes of such a persistent endothelial permeability may be considered (1) a continuous injury or one which is repeated at intervals which are too short in proportion to the severity of the trauma to permit a complete recovery between whiles; (2) imperfect recovery after a too prolonged or severe subjection to adverse circumstances such as cold; and (3) incomplete differentiation of recently formed endothelial cells which, in other words, fail to arrive at maturity. The newly developed bloodvessels of scar tissue and those of certain congenital nævuses, perhaps, may be regarded as instances of the last condition.

After any local inflammation the small bloodvessels which have been involved in the process will take time to recover, and will continue to be atonic and abnormally permeable until circumstances have allowed them to regain their normal state. Meanwhile they will be refractory towards various stimulations; for example, they will not contract in response to adrenalin or pituitrin.

Duke [1,2] described several cases of sensitivity of the

skin to special forms of irritation. In these instances exposure to the special irritant was followed by urticaria and whealing; and whatever the special irritant happened to be in a particular case—cold, heat, light, mechanical irritation—the elicitation of an active response was followed by a period of irresponsiveness in the affected area to further applications of the same stimulus.

Lewis, inquiring into the responses of the human skin, found that such a refractory period followed whealing caused by various stimulations, including the local application of histamine by pin-pricks, stroking the skin of individuals who exhibited factitious urticaria, freezing, burning, exposure to ultra-violet light, X-rays and γ-rays, painting with mustard gas in solution, and injecting antigen into a sensitised subject. Lewis observed, further, that in these refractory and reddened areas of skin the vessels no longer responded by vaso-constriction to direct local injections of adrenalin or pituitrin. This condition of irresponsiveness of the small vessels calls for some attention. In the first place, it is not difficult to conceive in a broad though perhaps indefinite way how endothelium, which has become so changed from the normal that solid particles can traverse its cytoplasm, may be unable to diminish the vascular calibre in response to adrenalin or pituitrin; for cytoplasm so altered might fail to exercise any considerable traction. Nor is it difficult to appreciate that a recovery of tone will require the lapse of time combined with favourable conditions, and that, following severe or oft-repeated injury, a restoration to normality may be for a long while, and perhaps indefinitely, delayed. Furthermore, it is part of the problem that the vascular endothelium is not neces-

sarily or usually the only seat of cellular damage; the trauma received by the extravascular cells may be even more severe, and until they are restored to health a complete recovery of tone in the neighbouring capillaries is unlikely to come about. A diminution of alkalinity or an increase of acidity is, apparently, the usual accompaniment of damage to the tissues, and as long as a condition of excessive acidity continues in a tissue it seems that any contiguous capillaries will be deprived of their normal tone, and their endothelium will for a corresponding period display an increased permeability.

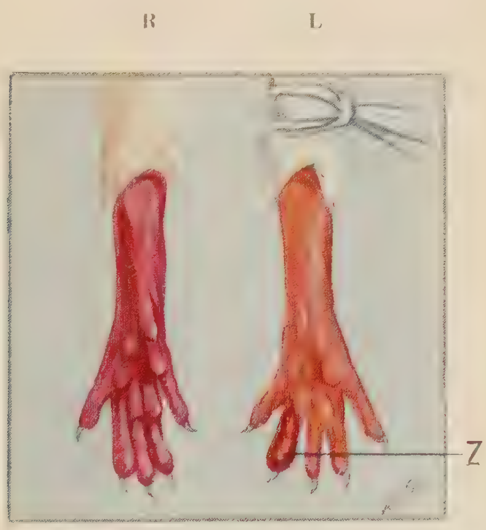
The failure of inflamed skin to produce wheals, though assuredly a fact, needs separate consideration. Whealing cannot be induced a second time in the same area of skin without the intervention of a more or less protracted interval of time. It appears to the author that this refractoriness may depend, not upon any degree of impermeability of the affected endothelium, but upon (1) a relative equilibrium established by the first effusion within and without the vessels concerned, and (2) in part, perhaps, on the fact that an inflamed tissue resists the entry of colloidal and other particles (p. 223).

In normal healthy conditions the balance of forces is such that any intervention which leads to a sudden increase of permeability of the capillary walls will lead to a more or less sudden effusion into the tissues of water and plasma from the blood until an equilibrium has been established. If the stimulus has been mild and of short duration, impermeability will soon be restored to the walls of the capillaries, the effused fluid will pass away through the tissue spaces

PLATE VIII

Relative acidity of inflamed tissue as indicated by vital staining.

A. A wheal (X) was made on the epilated skin of a rat, and phenol red was given intravenously. A skin incision (Y) was then made near the wheal. The colour of the wheal indicates an increase of acidity confined exactly to the lesion. No acidity has appeared in the edges of the skin incision.



Relative acidity of tissues following venous obstruction.

B. Before injecting phenol red the left hind leg was constricted so as to cause venous obstruction. A change of pH towards the acid side is shown by the colour. This change of hue is not obvious in the inflamed toe (Z).

and the lymph stream, and once again the local conditions will be in a state where whealing is possible.

It seems reasonable to suppose that when the vascular endothelium fails to recover and remains unduly permeable, either because the initial damage was excessive or for other reason, then it will become impossible for pronounced differences of osmotic, hydrostatic, and electrostatic forces to become created between the interior and exterior of the capillary walls, and consequently whealing will not occur. Accepting this explanation, it is not to be supposed that all transport between the blood and the tissues will be arrested during the so-called refractory period. The metabolic and other processes that create a raised electrical potential in the tissues are at work whether the capillary walls are unduly permeable or not, and consequently, as long as permeability continues, a slow seepage of electronegative colloids from the blood into the surrounding tissues may be believed to come about. Furthermore, such a process may be accompanied by the passage also of bacteria and other agents of disease, which, becoming established in the tissues, will give rise to a continuing inflammation, and so perpetuate the abnormal permeability of the vessel wall.

The fact stands out that an increased exudation from the bloodvessels takes place continuously in the presence of pyogenic infections or of other persistent causes of irritation, as, for example, the turpentine in a fixation abscess brought about by injecting that substance; whereas with non-septic and discontinuous traumatisms, periods of time intervene during which little or no exudation takes place from the bloodvessels into the injured part.

In view of the explanations here offered it would be well, in the author's opinion, not to include a failure to induce wheals among the phenomena of "irresponsiveness," but to confine this term entirely to a failure on the part of capillary vessels to respond by alterations of calibre to suitable stimuli. Used in this sense the word has a well-defined meaning inasmuch as it conveys to the mind the presence of a vascular condition which not only entails atony of the capillary wall, but implies an increased permeability also. This in turn confirms the presence for the time being of a *locus minoris resistentiæ* in the meaning given in the introduction to this work.

This condition of vascular irresponsiveness can hardly be regarded as one of health even though its presence is accompanied by a resistance to the formation of wheals in response to pathological assaults. Such an idea may be considered in connection with the treatment of patients suffering from peculiar sensitivity to specific stimuli. To maintain the capillaries of the affected region in a condition of atony by the administration of small doses of the specific poison may render the patient more comfortable by preventing any excessive and sudden inflammatory exudation; and because of the relief afforded such treatment may be justified. But it cannot well be regarded as a means of attaining a *restitutio ad integrum*, nor does the term "desensitisation" appear always to convey any profounder significance than the avoidance of acute exudative reactions.

We know too little about the precise changes which permit an abnormal degree of vascular permeability to be able to reverse the condition at will.

The first step would seem to be a removal of the cause directly responsible for the loss of vascular tone, such as exposure to cold, ultra-violet light, heat or other injurious agent. The next recuperative measure would seem to be a good supply of well-oxygenated blood. A circulation of this sort, by inference, may be accepted as causing or tending to cause a decrease of endothelial permeability. There is indirect evidence to support such a view (pp. 29, 62, 164).

The Inflammatory Response.

From some of the previous discussions it will be apparent that a high degree of impermeability of the vessel walls immediately before the onset of inflammation will lead to a copious exudation, while a lesser degree of impermeability beforehand (the phrase must be excused) will lead to a corresponding reduction of the subsequent inflammatory effusion. So far as the truth of these assertions is admitted, a test of the relative permeability of the vessels in a given tissue will be the amount of exudate poured out in that tissue in response to a given stimulus. This may be called the "inflammatory response," and, if so, must be distinguished from the remoter effects. Unless such a distinction be made, confusion is apt to arise in the interpretation of an experiment such as follows. Roger divided the sympathetic nerve of a rabbit's ear and then inoculated both ears with equal quantities of a culture of *S. erysipelatis*, and he found that erysipelas appeared much more promptly in the hyperæmic ear than in the normal one. Twenty-four hours after the inoculation the enervated ear showed, in addition to the redness and warmth due to vasomotor paralysis,

some œdema at the point of inoculation, whereas the other ear appeared normal or nearly so. In the following two or three days erysipelas developed in the two ears, the lesion being always more pronounced in the denervated ear. But from the third to the fifth day the appearances altered so that the ear with intact sympathetic nerves became the seat of a severe lesion, while the other ear had become almost normal. It is manifest that if, when describing such a result, a reference were made merely to the "inflammatory responses," the reader might be entirely misled as to the immediate and ultimate consequences of the induced hyperæmia.

In connection with the above experiment it may be recalled to mind that, in the presence of a persistent vascular permeability due to inflammation, hyperæmia causes an increased exudation of lymph.

Recently a series of papers has appeared by German authors who claim to show by experiment a relationship between the oxygenation of the blood and degrees of early inflammatory reaction. Briefly stated, the nature of this inquiry was as follows: The response evoked by the application of mustard oil to the shaved skin of a rabbit was noted, and its variations in severity, as displayed six hours later, correlated with the respiratory activities of the animals under different circumstances. The general theme of all these papers is to the effect that a diminished respiration leads to a decrease of hydrogen ions in the blood, an increase in the tissues, and a reduction of the inflammatory response of the skin to mustard oil.

Lipschitz found that antipyrin, which is a respiratory depressant, lessens inflammation, while pyramidon,

which is not a respiratory depressant, has no inhibitory effect on the inflammation. Peng noted that 0·250 gm. of urethane per kilo of body-weight given to a rabbit caused diminished respiration and a pronounced inhibition of inflammation without causing any material lowering of the body temperature. Guggenheim found that cardiazol increased both the respiratory frequency and the local inflammatory response; moreover, this drug counteracted the lessening of respiration and inflammation which ordinarily follows the use of urethane. Further, he observed that by artificial respiration he could counteract the antiphlogistic effect of urethane. Winckler found that sodium bromide given by the mouth in doses of 0·75 gm. per kilo reduced the frequency of respiration and the minute-volume of respired air in rabbits without lowering the body temperature, while it also lessened the inflammation resulting from applications of mustard oil. These effects were long lasting, but cardiazol given early in the experiment abolished the inhibitory effects of the bromide on both the respiration and the inflammation. Fröhlich sums up the matter as follows: Bromide, urethane and novanol all cause a decrease of breathing and a reduction of the early inflammatory response to a local irritant applied to the skin. Cardiazol stimulates breathing and counteracts the antiphlogistic effects. Urethane by itself produces an inhibition of inflammation which far outlasts its narcotic influence. The inhibition of inflammation Fröhlich attributes to an increase of the alkalinity of the plasma. He says that the CO_2 alveolar tension is definitely raised in animals under urethane and the CO_2 combining power of the plasma also is increased. The

hydrogen-ion concentration of the blood is distinctly lowered, and remains at an abnormally alkaline level for at least twenty-four hours.

These experiments appear to support the view that a full supply of well-oxygenated blood has the effect of increasing inflammatory exudation. The main factor, however, may have been a reduction of metabolic activity caused by the drugs which were used. Such a reduction would lead to an increased alkalinity in the extravascular connective tissues. Variations of pH, being much more readily effected and covering a far wider range in the tissues than in the blood, may be supposed on this account to be the chief agents in regulating the inflammatory response.

It is to be remembered that the term "permeability" as applied to the bloodvessels is not a term of scientific accuracy. Its use is one of convenience alone, and is often applied indiscriminately to any conditions in which permeation occurs. In fact, the permeation of the bloodvessels by colloids is dependent not alone upon the state of the vascular endothelium, but upon the relationship between certain conditions within and without the vessel walls. The chief of these, the writer believes, is the difference of electrical potentials as between the interior and the exterior of the capillary vessels. The more alkaline the blood, and the less alkaline the extravascular tissues, the bigger will be the difference of potential between the inner and outer surfaces of the capillaries; consequently the greater will be the resulting effusion if the plasma is allowed by some sudden physical change in the vessel wall to respond to the attraction of the positively charged tissues outside.

CHAPTER XVI

SOME THERAPEUTICAL CONSIDERATIONS

AMONGST the material collected in previous chapters are some observations concerning the effects of hyperæmia, inflammation, venous congestion, and immobilisation, which have a bearing on therapeutics. These may be used as the basis for a brief discussion.

1. THE CURATIVE USE OF HYPERÆMIA.

From a remote age until today measures which increase a local blood flow have been used in the treatment of disease. Hippocrates employed fomentations and placed the actual cautery amongst the most potent of remedies. The modern physician may be inclined to replace the hot iron by some milder application; otherwise his principles hardly differ from those of his ancient masters. Diathermy, fomentations, dry heat, tincture of iodine and other chemical rubefacients, and liniments are at one end of his list, while setons, blisters, fixation abscesses and cauteries are at the other. The general appreciation of these therapeutic agents and the selection from them of the one most suitable for an individual case, up till recent times have both rested almost entirely upon empiricism, tradition, and convenience. " To secure a good blood supply " to the diseased or injured part probably would cover the aim of most practitioners in the past. Today more discrimination is required, for, as will be seen, a copious blood supply is far from desirable in certain cases,

while in others a mere hyperæmia by itself will be insufficient.

Hyperæmia, in the absence of inflammation or other condition leading to an increase of endothelial permeability, has little or no effect towards accelerating the lymph flow or bringing about the diapedesis of white corpuscles (p. 24). Warmth in such a case may give relief of pain and be useful to this extent, but the curative benefits of hyperæmia will be small and, possibly, negligible. In the presence of an increased vascular permeability already established hyperæmia becomes a remedy of great value; by its help both exudation and diapedesis will now be accelerated and enhanced. For treatment a sharp and primary distinction must therefore be drawn between measures which bring about hyperæmia alone and those which induce inflammation. Their fields of utility are different, and conditions which demand the one may not require the other.

Apart from the supply of a full stream of well oxygenated blood, the main therapeutic purpose of hyperæmia is to increase the processes of exudation and diapedesis when these are already present, for, as remarked above, it cannot initiate them to any material extent. Generally speaking, in the presence of a local inflammation, the small vessels in the tissue concerned will be atonic and their walls will be readily permeable. To increase the facilities for transudation and diapedesis in the affected part by such non-irritating measures as fomentations will be the clinician's aim in these circumstances, and seldom will there be reason for using a more drastic treatment with the object of causing thereby an additional in-

flammation. Only when it is desirable to induce a durable result by a single application—as may be the case in occasional and exceptional circumstances—will it be advantageous to use blisters or, possibly, the cautery to enhance the blood flow in a part already inflamed.

Some experimental evidence indicating that inflammatory exudation is favoured by measures which increase the local blood flow has been set out already (p. 251). Further evidence of the same sort follows. Danilewski tested the effect of hyperæmia in rabbits' ears on the healing of wounds and on the course of inflammatory lesions. The cervical sympathetic nerve on one side having been divided, small pieces of skin were removed from both ears by punch forceps. Healing occurred earliest in the ear whose sympathetic had been divided. In other rabbits, hyperæmia having been caused in one ear by the same method as in the previous experiments, inflammation of both ears was caused by applications of croton oil. Danilewski found that the inflammatory reactions were accentuated in the hyperæmic ear, but healing was accelerated and more often occurred without loss of tissue. With irritation sufficient to cause blistering, the exudate was always more copious in the hyperæmic ear, but the pus which formed was *pus bonum et laudabile*, whilst that in the lesions of the other ear had a grey tinge, and was watery and transparent. After more intense inflammation necrosis occurred only in the ear whose sympathetic supply was intact. Furthermore, irritation which was too weak to cause visible responses in a normal ear would cause reactions in a hyperæmic one.

Samuel carried out somewhat similar tests on

rabbits. He divided the auricular nerves on one side and the cervical sympathetic on the other, and then subjected both ears to water at 54° C. In the hyperæmic ear acute inflammation with rapid recovery ensued. In the ear whose vessels were constricted as a result of division of the auricular nerves a relatively slight vascular dilator response occurred in the vessels and was confined sharply to the injured tissue, recovery was delayed, and gangrene was apt to supervene.

Apart from the confirmatory opinion as to this benefit from hyperæmia, which seems to be held generally by clinicians, a specific example in man which has almost the quality of an experimental demonstration is provided by the old-fashioned treatment of erysipelas. This consisted of painting the spreading edge and the healthy skin around it with tincture of iodine.

The next point is that an artificially induced hyperæmia is not to be regarded as a panacea for local infections. There are certain conditions at least in which it should be avoided, or at any rate not employed without a full knowledge of what the consequences may be. The first of these is a freshly made wound in which, judging by experimental work with dyes, dissemination from the affected tissue is likely to be accelerated in the presence of hyperæmia. In dealing, for example, with a finger punctured during the performance of a post-mortem examination, it would appear undesirable to encourage hyperæmia in the wound until a sufficient time has elapsed for the development of those reactionary conditions which will cause a local fixation of any bacteria that may have been introduced. Admittedly this statement lacks a

foundation of direct bacteriological experiment, which is unfortunate in view of the issues involved; but it has a reasonable basis in analogy, on which it must stand until additional data have been acquired in the laboratory. For the great danger in a case such as that which has just been supposed is septicæmia, and there is reason to fear that the dissemination of micro-organisms from a fresh wound surface will be facilitated by hyperæmia. Okuneff's observations upon the comparative rates of absorption of trypan blue from subcutaneous tissues in the presence and absence of hyperæmia must be considered significant in this matter (p. 211).

Another condition in which hyperæmia, so far from being instituted, must be avoided, is tuberculosis. When the writer was a student it was commonly taught that tuberculous disease of the hip-joint was as fatal as cancer. Since the pioneer work of Thomas and his successor, Robert Jones, who taught that tuberculous joints must be rested by rigid splinting continued over long periods, the cures in uncomplicated cases have risen to a number approaching 100 per cent. provided that the required standards of treatment and after-care are available. It would be going too far to attribute such a revolution in prognosis entirely to the avoidance of that copious blood flow which is the inevitable accompaniment of activity, but it can hardly be doubted that the prevention of hyperæmia has played a large part in the improved prognosis. There is abundant clinical evidence from sources other than tuberculous bones and joints to indicate the malign effect of hyperæmia upon the growth of the tubercle bacillus in living tissue. The less hopeful outlook for

any tuberculous lesion, once it has become infected by other bacteria which maintain a local hyperæmia, is generally recognised, and so too is the bad prognosis of phthisis when imposed upon an otherwise uncomplicated pulmonary silicosis. And so far from treating a tuberculous adenitis or arthritis with fomentations, the modern clinician would look askance at the suggestion. With the possible exception of exposure to direct sunlight, in which presumably a general effect upon the organism is aimed at, it may be said that the successful management of these cases includes the avoidance of excessive warmth, and of movements and other agencies which are likely to bring about an increased flow of blood in the affected part. Even when we consider the exposure to sunlight we must admit that, speaking generally, those patients prosper best whose exposed skin becomes pigmented most readily, and who thereby are most protected from a hyperæmia consequent upon the exposure. And it may be questioned whether the others would not be assisted toward recovery by cutting off the heat and light rays from the affected part while still permitting exposure to the cooling airs.

The foregoing remarks may seem extravagant in view of the fact that Bier's "hyperæmic" treatment arose from a study of tuberculosis of bones and joints. Yet they seem not to be irreconcilable with Bier's own views.

Meyer and Schmieden, in their exposition of *Bier's Hyperæmic Treatment*, state that, "*In the treatment of surgical tuberculosis obstructive hyperæmia only, by elastic bandage or suction glasses, is used; never hot air.*" The italics are theirs. They advise that the con-

striction should be enough to cause slight cyanosis of the part under treatment.

Much misunderstanding of Bier's work in this country probably is due to a fault of nomenclature. In our surgical writings the term "hyperæmia" is often applied, without any discrimination, equally to active vascular dilatation and to venous obstruction—two conditions which are dissimilar as regards both cause and consequence. In these pages the term "hyperæmia" denotes only an increased flow of blood.

Reverting to the main theme, that is to say the harmful effects of hyperæmia upon tuberculous lesions, an explanation of the fact is perhaps provided by some experimental work carried out by Corper, Lurie, and Uzei. These inquirers had noticed that whether introduced into guinea-pigs, rabbits, dogs, or monkeys, the largest tuberculous lesions were always formed in the lungs. The liver, in contrast with the lungs, offered a high resistance to this infection. It occurred to these workers that, while the alveolar air has the highest oxygen tension and the lowest carbon dioxide tension of any internal part of the body, in the liver there is a particularly low oxygen tension and a high content of carbon dioxide. Accordingly they carried out an investigation into the comparative growths of tubercle bacilli when placed in artificial media and subjected to varying concentrations of oxygen and carbon dioxide. They found that the effect of different concentrations of carbon dioxide could be regarded as negligible as a factor in tuberculosis. On the other hand, within certain limits, the rate and luxuriance of the growth of tubercle bacilli were directly proportional

to the oxygen tension of the gaseous environment. *In vitro* the bacilli grew better in an atmosphere of alveolar air than in one of the gases of mixed venous blood, and in an atmosphere of 0·1 per cent. oxygen they failed to grow at all. Referring to the living body they remark that conditions which can change the oxygen tension in tuberculous foci from 0·7 mm. to 3·8 mm. may be sufficient to change a quiescent into a progressive lesion. Up to a point these observations seem to be in agreement with the experience of most clinicians who habitually appear to regard hyperæmia as a condition to be avoided in the treatment of so-called surgical tuberculosis, and who deliberately treat tuberculous lesions of a lung by bringing about collapse of the affected organ.

Another infection which may perhaps find hyperæmia favourable to its development is syphilis (p. 91).

Further knowledge on this wide subject, and especially as it bears on the spread and pathogenicity of viruses, is greatly to be desired.

2. The Therapeutic Value of Artificially Induced Inflammation.

Inflammation may be induced as a therapeutic agent for any of the following purposes: (*a*) As a means of producing an accessory hyperæmia in the neighbourhood of a diseased focus; (*b*) to forestall the establishment of infection in a freshly inoculated healthy tissue; (*c*) to prevent the spread of established infection by the creation of an antibacterial barrier around the focus; and (*d*) to bring about the local fixation and destruction of micro-organisms which are free in the circulating blood. To these may be added, perhaps,

(*e*) the localisation of pharmaceutical remedies within diseased tissues.

(*a*) *The Production of Accessory Hyperæmia in the Neighbourhood of a Diseased Focus.*—The remark has already been made that with lesions accompanied by an increased permeability of the bloodvessels an additional hyperæmia induced by fomentations and suchlike mild therapeutic measures will be sufficient usually for our purpose of assisting nature. But there are cases in which a method which results merely in the production of local hyperæmia will be insufficient. Two examples may be suggested. In the one there is a lesion unaccompanied by any considerable increase of vascular permeability; in the other, although the vessels have already been rendered more permeable than normal, yet, owing to peculiar circumstances, it is desirable to bring about a long lasting accessory hyperæmia by means of a single application. In both of these instances the use of a blister or the cautery or some other inflammatory agent may be demanded rather than a milder remedy which will cause a mere temporary hyperæmia unaccompanied by inflammation.

(*b*) *The Prevention of Infection in a Healthy Tissue.*—Could we always be forewarned of the exposure of a healthy tissue to infection it would be possible, by inducing a moderate local inflammation beforehand, to raise greatly the powers of resistance in the threatened area. The general method by which such enhanced resistance can be effected has been discussed at sufficient length in an earlier chapter (p. 231). In practice little use seems to have been made of this therapeutic weapon, although a definite field for its utility seems to be offered. For example, it is recognised that

tissues which have been submitted to heavy radiation with X-rays are liable to show a great reduction in their resistance to infection, and this is a matter which the surgeon has to bear in mind if called upon to operate upon a part of the body where such a state is present. In a case of this kind it would seem feasible to reduce the hazards of infection by initiating an aseptic inflammation so as to secure an increase of the macrophages within the zone of the intended operative interference—for the loss of resistance to infection may reasonably be attributed in part at least to a deficiency of macrophages owing to their destruction by the exposure to X-rays (see Mottram).

(*c*) *The Prevention of the Spread of Sepsis from a Focus of Infection.*—Under this heading we have to consider two conditions—namely, (i.) the introduction of pathogenic organisms into previously healthy tissue with the danger of their dissemination through the body, and (ii.) the gradual spread by direct extension of an established focal infection.

(i.) As mentioned already, a pre-existing, non-destructive inflammation renders the affected tissues more than normally resistant to subsequent infection. This is not the only beneficent consequence of inflammation, for, in addition, it has a potent effect in preventing any wide distribution through the vascular and lymphatic channels of bacteria which have entered the body and have maintained their vitality and capacity for harm. In ordinary circumstances micro-organisms introduced into healthy tissues rapidly find their way into the lymph and bloodstreams (p. 216). Such dissemination does not come about when bacteria are injected into an inflamed tissue. There is reason

indeed for supposing that once the inflammatory reaction has become well established the only common way in which general dissemination occurs is by the detachment of emboli from infected clot within a vein. That is to say, the ordinary means of distribution of sepsis, as it occurs from healthy tissues, is almost, if not entirely, prevented by inflammation.

In those cases where dissemination of sepsis comes about in the later stages of an infective process the usual cause is a septic phlebitis. Evidence bearing on this was collected by the author during the Great War, for he found in a hospital in France away from the front line that whilst septicæmia was relatively rare, septic phlebitis actually appeared to be the most frequent cause of death following gunshot wounds of the limbs. Thus among twenty-five such fatalities occurring consecutively in which examinations were made post-mortem, septic phlebitis was noted in ten, and pulmonary infarcts were found in another four. That is to say, acceptable evidence of phlebitis was present in just over one-half the total number. In yet another seven of these cases broncho-pneumonia existed, and it is conceivable that this condition in some at least may have been due to septic infarction.

While considering this subject of the prevention of sepsis in healthy tissues by setting up a local aseptic inflammation, the use of antiseptics as applied to freshly inflicted wounds calls for some consideration. The position appears to the author to be as follows: Lister proved that by the free application of certain chemical antiseptics to wounds the danger as it then existed from sepsis could be much reduced. A few modern theorists have objected that some of the

commonly used antiseptics do not destroy bacteria in wounds, and, moreover, are irritants and therefore should be avoided. The weakness of their view—putting aside the greater value of practical experience as compared with arguments—seems to lie in the two assumptions (1) that the only beneficial use of an antiseptic is as a direct germicide, and (2) that irritation of a wound is harmful. But it is conceivable that antiseptics act in other ways than by a direct destructive action on bacteria, and it is far from certain that irritation of the surface of a wound—except, perhaps, in certain special tissues—is harmful. Apart from any bactericidal effect, a slight inflammation will almost certainly tend to prevent the spread of sepsis. Moreover, it may be that the coagulating effect on protein of most recognised antiseptics will impede the immediate dissemination of bacteria—which otherwise is to be expected in a freshly made wound—until the inflammatory reaction is sufficient of itself to effect fixation. The application of irritating chemicals to a wound surface would seem certainly to provide that aseptic inflammation which is such a potent defence against infection—provided, of course, that the effect of the irritant stops short of actual destruction of tissue.

The temporary irritation caused to wound surfaces by antiseptics, so far from being inimical to healing, may favour the process. Carrel, as the result of experiments on dogs, concludes that irritation expedites the healing of skin wounds, and that the onset of reparative processes is delayed in a wound which is not irritated in any way. Unpublished observations by the author seem to confirm this view.

In the case of freshly inflicted wounds it would

seem desirable, if such a choice be possible, to use antiseptics which do not increase the initial hyperæmia present in consequence of trauma. But the writer is not sufficiently informed to discuss this matter in detail. Once inflammatory fixation has become established in a wound, the application to it of antiseptics or other substances of a slightly irritating nature would appear to be advantageous on the general principle that exaggerated hyperæmia is an accessory curative agent in the presence of inflammation.

(ii.) The most efficacious method of opposing the spread of bacteria in living tissues is to set up in these tissues a preliminary inflammation of such a nature as to cause a concentration of macrophages. To create such a barrier thirty-six or forty-eight hours will be required. In other words, the infection must be forestalled. Nevertheless, such anticipatory treatment has its place in surgery. For example, in the case of a streptococcal or any other infective empyema which is to be treated by airtight suction drainage, it is usual to perform aspiration at the first exploration. When this is done the chest wall in the region of interference can be infiltrated with sterile broth so as to set up a local aseptic inflammation. By this means the risk of cellulitis developing around the subsequent drainage wound may be much reduced if not entirely eliminated. Such at least is the teaching of laboratory experience (p. 231).

Roeder, remarking that with virulent, non-suppurative bacterial invasions incisions do more harm than good, recommends the following procedure for use in cases of severe sepsis in the limbs. He chooses a spot 4 or 6 inches above the apparent upper limit of

the ascending infection, and at this spot separates the muscle bundles from one another and inserts strips of gauze. This sets up an aseptic inflammation which checks the spread of infection beyond. Though his procedure is based on correct pathological principles, it may be thought that an equally efficient antibacterial barrier might be effected without causing so much anatomical disturbance.

(*d*) *The Local Fixation and Destruction of Micro-organisms Present in the Circulating Blood.*—Not only does inflammation prevent dissemination of infection, but it may have a remedial value once such dissemination has occurred, inasmuch as an inflammatory focus will segregate micro-organisms from the bloodstream. This perhaps may be the reason, in part at least, why, with the appearance of inflammatory foci, the bacteria disappear from the blood in pneumonia and typhoid fever. The fact itself represents the principle underlying the treatment by fixation abscess, cautery, or other therapeutic measures which involve the establishment of an inflammatory focus for the cure of a general blood infection. Moreover, matters other than bacteria can be localised by an abscess which, indeed, may be looked upon as a temporary organ whose function is the elimination of noxious agents from the blood.

Although the curative effects of cutaneous inflammation appear to have been recognised empirically from the dawn of history, they receive relatively little attention today. The supposition arises that they are neglected because their mode of action has not been understood. Jenner, in a letter to a colleague, which has been quoted elsewhere (p. 113), not only expresses his own belief in the therapeutic value of cutaneous

irritation, but makes it clear by references that a similar opinion had been expressed by some of his contemporaries and predecessors. His explanation is that an inflammation of the skin has the effect of diverting disease from other parts of the body.

In smallpox, Jenner thought that with the appearance of vesicles "the disease, as far as it depended on the *primary action* of the infectious matter which called it into existence, terminates." A new train of symptoms follows on account of the pustules. He regarded the secondary fever in smallpox as different from the primary, and believed it to be amenable to treatment of the pustules with suitable chemicals.

Clearly Jenner had little doubt that the agents of diseases became localised by the influence of inflammation. It is of human interest to note that although the accuracy of his observation has since been confirmed, the practice which he based upon it is still in need of advocacy. This is the more surprising in that the only known method of localising bacteria from the bloodstream is inflammatory fixation. There is no alternative measure.

Laboratory work has shown that if bacteria are injected into the bloodstream of an animal in which an abscess has been induced, some of the bacteria will find their way into the abscess. This fact may be regarded as confirming the judgment of Jenner, Fochier and others in causing cutaneous irritation and fixation abscesses for the treatment of mankind. It must, however, be noted that these methods are not generally viewed with enthusiasm by clinicians; and there can be little doubt that a much fuller knowledge is required as to certain details which may influence the

results. For example, the readiness with which particles undergo diapiresis appears to depend largely on their size. A mustard bath which may be sufficient to localise viruses in the skin may yet be too mild to fix bacteria owing to their greater dimensions. For the localisation of these a mustard poultice may be more effective than the less vigorous though more extensive stimulus of a mustard bath. Evidently much experimental work must be carried out before a high degree of therapeutic success can be expected from this form of treatment.

Not all who have put the affair to a practical test in the laboratory are satisfied as to the alleged protective influence of a fixation abscess against a general blood infection. Le Guyon, for example, carried out a number of experiments on animals and failed to convince himself that a fixation abscess had any effect at all in combating the diseases which he artificially induced. Guinea-pigs were inoculated with anthrax and half of the total number immediately after the inoculation were given a subcutaneous injection of 0·4 c.c. of turpentine to initiate an aseptic abscess. The disease ran a similar course in all the animals, and death was not delayed in those with subcutaneous abscesses. Similar tests were carried out upon mice inoculated with virulent streptococci and pneumococci, with guinea-pigs poisoned with diphtheria toxin, with rabbits and guinea-pigs infected with tubercle. In none of the experiments was the fatal termination delayed or prevented by a fixation abscess. It may be that Le Guyon put the method to an excessively severe test. Nobody will suppose that a fixation abscess can segregate all the micro-organisms circulating in the

blood. The liver and spleen will still retain a large proportion. Nevertheless, if given sufficient time, a fixation abscess will take and hold some of the circulating bacteria, and by so much may be supposed capable of relieving the assault made upon the reticulo-endothelial organs; and not only does a fixation abscess collect bacteria in this way from the blood, but it also destroys them.

(*e*) The use of inflammation for the purpose of localising pharmaceutical remedies calls for little discussion in this place. The subject is one that awaits exploration in the laboratory. A reference to the subject based on hypothetical and observational grounds appears on p. 276.

It seems possible that some of the supposed selective affinities between certain tissues and particular drugs—as, for example, that of tumours for lead (Blair Bell) and salvarsan for the lesions of syphilis or for the treponema itself (Ehrlich)—are in reality instances of localisation under the influence of inflammation.

3. Venous Congestion.

The consequences of obstructing the venous return from a tissue involves a rise of hydrostatic pressure in the capillaries distal to the point of blockage, and if the endothelium of these capillaries has already been rendered abnormally permeable by inflammation or any other cause, this rise of hydrostatic pressure may enhance the process of exudation from the bloodstream (p. 26). Even in the absence of any pre-existing affection, venous congestion, if of sufficient degree and maintained for a sufficient length of time, will of itself increase the permeability of the capillary endothelium.

Such an effect is in some ways the opposite of that resulting from hyperæmia.

Theoretical considerations suggest that the results of venous congestion may be of value therapeutically in certain conditions, and particularly in tuberculosis (p. 260). At the same time it has to be borne in mind that if, after a period of venous congestion, the obstruction to the blood flow be suddenly removed, a reactionary hyperæmia will follow. The suggestion is that when treating tuberculous lesions by congestion the hindrance to the blood flow ought to be long-continued in order that the period during which the affected part is subjected to reactionary hyperæmia is reduced to the minimum. Another use for venous congestion will be proposed when the treatment of freshly inflicted wounds is under discussion.

4. Immobilisation.

Attention has been called by many laboratory experiments to the very slow passage of lymph in a healthy limb at rest, and to the large increase of flow under the influence of movement. A reference will be made later to the obvious bearing of this fact on the dissemination of bacteria.

5. The Treatment of Infected Wounds.

To co-ordinate the foregoing principles into a plan of action it seems worth while to consider their practical application even if this entails some repetition. From what has gone before it will be clear that when treating local sepsis our objects and the means adopted for obtaining them will vary according to whether (i.) we are dealing with an infected wound in the earliest stage

—that is to say, before inflammatory fixation has become established—or (ii.) with one which is already both inflamed and infected.

(i.) Consider first a perfectly fresh wound into which bacteria have entered. From such a wound any micro-organisms present may be expected to spread along the tissue spaces and lymphatic channels with rapidity in the first hour or two—that is to say, in the pre-inflammatory period. The clinician's immediate aim, therefore, will be to check such spread, if possible, until there has been sufficient time for that inflammatory reaction to come about which will itself prevent a further dissemination of the infection. The following suggestions towards achieving this aim admittedly rest on hypotheses derived somewhat indirectly from laboratory experiments. But seeing what a strong guidance is given by these experiments, and in the absence of more specific investigations, there is justification for tentatively applying the proposals to clinical practice.

The employment of some effective antiseptic whose utility has been proved by experience takes the foremost place in this preliminary treatment. We need not stop to discuss the method of action, to which reference has been made already (p. 265), except to suggest that the substance applied should be a coagulant of protein, and that it should cause a minimum amount of pain and hyperæmia. Carbolic acid appears to have these qualities. Unfortunately, it is not possible to discuss this matter in scientific biological terms, because the investigations carried out with a view to determining the value of antiseptics as applied to wounds has been based principally upon their bactericidal efficiency

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in vitro instead of upon their effectiveness *in vivo*, and the importance of other qualities than mere bactericidal action has been largely overlooked.

Immobilisation of the wounded part—which may involve immobilisation of the wounded individual—is the next important requirement. The fact that almost no lymph flows from a limb at rest, while movement at once produces an active flow, is a sufficient indication for keeping at rest the wounded part until an inflammatory reaction has become established sufficiently well to retain any bacteria present within the immediate vicinity of the wound. With the same principle in view any manipulation of a recent wound must be of the gentlest.

Another effect of rest will be to avoid some of that early hyperæmia which is suspected of accelerating the dissemination of bacteria in the initial stage of infection (p. 258). Other causes of a premature hyperæmia in the neighbourhood of the wound, such as soaking in hot water, also should be avoided for the same reasons.

Moderate constriction of a wounded limb so as to produce some cyanosis appears to be another desirable measure at the beginning of a local infection, for this will hinder the return flow of lymph and blood. These precautions, it must be understood, are suggested with the reservation that they need the support of controlled experiment before their value can be correctly estimated.

(ii.) Once an infected wound has become inflamed, septicæmia, if it has not already arisen, almost ceases to be a danger, and hyperæmia will now be the prime remedy, for by its means exudation and diapedesis will

be much accelerated. In later stages, if pus has formed, it must be provided with an exit, but even here laboratory observations, somewhat indirectly, lead to the view that incisions for the drainage of pus should encroach as little as possible on healthy tissues. To cut through the inflammatory barrier and put the infected tissue in direct communication with uninflamed tissue is a calamity to be avoided wherever possible, and when it has to be done the opening up of uninflamed tissue should be of the smallest extent compatible with effective drainage. Large incisions to drain abscesses and to allow them to "heal up from the bottom" ought to be regarded as based upon fallacy. It is difficult to perceive the need for any opening larger than is required to drain the wound and so to relieve tension. Small openings are compatible with good drainage, provided that they are not plugged with gauze or other material and are kept open by a tube or efficient substitute.

The prime object of opening an abscess is not to let out the pus, but to prevent tension and its consequences of toxæmia and of a local extension of the bacterial invasion. Pus which is free to escape need not be feared as a possible cause of the local spread of infection. In circumstances of free drainage pus is bactericidal, and may be regarded as truly laudable.

As in the case of a freshly infected wound, so also in the later stages when an abscess has formed, it is undesirable to apply any undue stresses to the diseased focus lest the barriers which nature has set up against the spread of infection be broken down. The perennial temptations to elicit fluctuation when diagnosing an abscess and to squeeze out pus when we are opening

one must always be resisted. The use of dressings which adhere to the wound surface so that the granulations are injured with each change of dressing would also seem to be undesirable.

Where a sufficient inflammatory barrier does not already exist, an artificial one may be created, as mentioned earlier (p. 231), provided that in the peculiar circumstances of the case a delay of thirty-six hours or so is permissible.

In the presence of septicæmia, especially if the wound of entry is small, as it often is, the possible value of fixation abscesses calls for attention, because there is no doubt that such abscesses segregate bacteria from the blood; moreover, an active focus of inflammation seems to offer the only means available for such a purpose. It would further appear that once established the efficacy of a fixation abscess might be amplified by accessory hyperæmia induced by fomentations.

6. Localisation of Remedial Agents.

The last point which arises from the facts collected in the earlier pages of this book is the question whether it may be possible to utilise our knowledge of the localisation of substances from the bloodstream in order to introduce remedies effectively to the sites of disease. It has been shown that colloidal substances with particles which are not too large and which carry a negative electric charge, when injected into the bloodstream, will with certainty escape from the bloodvessels in an inflamed tissue and, reaching the extravascular regions, will be retained there in a relatively high concentration. In view of the fact that a sterile

inflammation can be set up at will in any part of the body, it seems almost certain that by this method we can localise any substance that can be prepared in the necessary physical state in whatever organ we wish by introducing it to the bloodstream. Its deposition in the tissues, it is true, will largely be within the macrophages, though not always entirely so. Even in this position a medicament may be effective. It has been shown that the giant cells of tubercle take up electronegative colloids, and this, in itself, is suggestive (p. 58).

One striking example of fixation of a curative agent —air-borne and not blood-borne in this instance— appears to be afforded by the condition known as anthracosis. The matter stands thus: a man who has a deposit of silica in his lung is extraordinarily liable to develop phthisis, which usually progresses rapidly to a fatal ending. There is an exception to this rule, for the increased liability to tuberculosis among those who suffer from silicosis does not apply to coal-miners. In fact a coal-miner, as Cummins has shown, in spite of his exposure to silicosis, is less likely to die from pulmonary tuberculosis than is the average citizen of the same age. The following figures are taken from Cummins's paper:

Mean Annual Tuberculosis Mortality per 10,000 at Age Forty-five to Fifty-five.

All occupied males and retired civilian males ..	166
Coal hewers and getters	114
Metal grinders	1,149
Tin and copper miners	1,744

In other words, silicosis without anthracosis leads to a very heavy incidence of phthisis, while silicosis with anthracosis leads to an actual reduction of liability

to phthisis to a level below that which applies generally and apart from silicosis.

Anthracosis itself is dependent upon silicosis, the coal dust being retained in the lung owing to inflammation set up by the presence of silica. Coal dust alone causes little or no irritation, and becomes deposited in relatively small amount in a healthy organ.

In this instance we appear to have an example of (1) the lodgment of a noxious agent (*B. tuberculosis*) in the lung as a consequence of inflammation caused by the presence of silica, and (2) the fixation of a remedial agent (coal dust) in the same tissues by the same cause, namely, silicosis. This localisation of a remedy by means of inflammation is a field of therapy which awaits exploration, as suggested by the writer some time ago.

It seems that we can obtain a special concentration of any substance we choose in an inflamed tissue provided that we introduce it to the bloodstream in the form of an electronegative colloid whose particles are of suitable dimensions. As a rule there will be no great difficulty in effecting the necessary aseptic inflammation if it is not already present.

GENERAL SUMMARY AND CONCLUSION

THREE conditions are required for the localisation of many blood-borne diseases—namely:

(1) An abnormal permeability of the walls of the small bloodvessels.

(2) The presence of forces which will transport the noxious agents through the endothelial cytoplasm.

(3) The retention of noxious agents in the tissues under the influence of inflammation.

The three conditions mentioned above lead to the localisation, not only of the agents of disease, but to the factors of defence.

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